Vol. III, Nos. 2 and 3 January, 1917

THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
LONDON: FETTER LANE, E.C.
EDINBURGH: 100, PRINCES STREET
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C.
WILLIAM WESLEY & SON, 28, ESSEX STREET, LONDON, W.C.
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
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The Association of Economic Biologists

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VOLUME III JANUARY, 1917 Nos. 2 and 3

A BACTERIAL SPOT OF CITRUS.

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(With Plates III—XIII).

IN October 1914, toward the close of the Citrus season, a grower in the Western Province submitted for examination some lemons badly disfigured by dark-coloured sunken spots; he stated that he had first noticed this blemish two seasons back, but that it was rapidly on the increase; as much as 30 per cent. of his lemons were affected, and the trouble was spreading to the Washington Navel oranges.

No fungus mycelium could be found in the discoloured tissues, but there were pockets of disintegrated cells filled with innumerable bacteria. A pure culture was obtained at once from these tissues, and a few successful inoculations carried out. It was too late in the season however to enter on a complete investigation of the trouble, and further work in connection with it had to be deferred until the present year, when the matter was taken up as soon as favourable weather conditions started the spread of infection to the new crop of fruit.

A detailed study of the causal organism has now been carried out, and a survey made of orchards in the infected area with a view to preventing if possible a further spread of infection.

LITERATURE.

Two bacterial diseases of citrus have been described, both occurring in America; the so-called "citrus canker" of Florida need not be more than mentioned here; the macroscopic characters of the "canker" are entirely different from those of the spot under consideration, it is characterised by a proliferation and subsequent suberisation of the cells of the affected tissues (4).

A much more closely related trouble however is reported from the lemon growing sections of Southern California (6); it has been

appearing occasionally for the past three years, has gradually increased and is now assuming some economic importance. This disease has been named "Black Pit of Lemon" a term which aptly describes its effects on the fruit, where it causes circular-oval discoloured spots 5 to 20 mm. in diameter, the tissue being depressed somewhat below the bottom of the normal oil glands into the white portion of the rind.

The cause of the disease proved to be an actively motile rod with a single polar flagellum, and it has been named *Bacterium citriputeale.* The description and illustrations of fruit attacked by this organism correspond very closely with those of spotted lemons from the Western Province, and it was surmised that the same organism would prove to be the source of the trouble. A thorough investigation however has shown that the organism causing the disease in South Africa is quite distinct both in morphology and in cultural characters.

Distribution.

The Botanical Division of the Agricultural Department has no officer stationed within easy reach of the citrus orchards of the Western Province; arrangements were therefore made with the co-operation of Mr C. W. Mally, the Cape Entomologist, to systematically inspect the citrus trees in the districts with a winter rainfall, but unfortunately the exceptionally rainy season prevented this plan from being carried out with any degree of thoroughness. A number of orchards however, have been inspected and a large number of specimens of suspected fruit sent to Pretoria for examination; and a certain amount of information has been gleaned as a result of circularising the principal citrus growers of the Union.

The disease is now definitely known to occur on a number of farms in the region of Simondium and Lower Paarl. It extends along the Berg River Valley and has been found occurring at Wellington, Sir Lowry Pass and French Hoek. It has also been reported as occurring at Stellenbosch, but no specimens of supposedly infected fruit from that locality have been examined.

So far as can be ascertained the Transvaal is entirely free from the trouble; there is certainly no sign of it at Warmbaths or Nelspruit, and growers at Barberton state that during a careful examination of their trees they failed to find anything at all resembling the bacterial spot. The Rustenburg area also seems to be clean; one box of oranges and lemons sent from there in response to enquiries proved

to be attacked by anthracnose (*Colletotrichum gloeosporioides*) which causes discoloured spots on the fruit not very widely differing in appearance from those caused by the bacillus. They are different in colour however, not conspicuously sunken, and can be readily distinguished when the pinkish spore pustules begin to develop.

During November and December of this year (1915), after this article was practically completed, Mr Pole Evans, the Chief of this Division, made a personal inspection of the orchards in the infected areas. At that time there was little or no fruit on the trees so that his inspection resolved itself into a search for the disease on branches of trees and nursery stock.

Branch infections were very plentiful in the orchard at Simondium where the disease was first detected, in an orchard near Cillie's Siding, and one in Zuider Paarl: at Simondium the organism attacks stems ½ to 1 inch in diameter. At the Elsenburg Agricultural College, the nursery stock has been severely attacked but no signs of the trouble were found in any of the other nurseries which were inspected.

He saw no evidence in the orchards of the Clanwilliam, Piquetburg or Montagu Districts. It is apparently confined at present to the valleys of the Groot and Klein Drakenstein and the Berg River Valley; in this area the fruit has been attacked on a number of farms, but branch infections have only been detected in the localities mentioned above.

From field observations it would appear that the leaf is attacked first and that the disease is then communicated to the stem through the leaf stalk. As soon as the petiole and neighbouring stem tissues are invaded the leaf falls, and this would account for the fact that very few leaf infections were found on branches sent for examination.

SIGNS OF THE DISEASE.

The disease has been found occurring in nature on lemons of the Mediterranean type, navel oranges and naartjes, the term "naartje" including both Mandarins and Tangerines. It has been induced by artificial inoculation in lemon, orange (three varieties), naartje, shaddock, grape fruit, citron and sour and sweet limes.

In describing the appearance of affected parts, and the cultural characters of the organism, the colours named have been compared as accurately as possible with those in Ridgway's *Color Standards and Nomenclature* (5).

It was first noticed in Geneva lemons, on which it forms discoloured sunken spots varying from 1 to 3 mm. in diameter: more frequently

they are 5—10 mm. in diameter and depressed 1—2 mm. below the surface of the healthy rind. The spots are more or less round, but when a number occur in close proximity to one another, they coalesce and result in the formation of large irregular blotches (Plates III and IV).

A close examination of the spot usually reveals a small wound in the centre which has been the starting point of infection; in a few cases a large diseased area showed numerous scratches, possibly the result of swinging against a thorn in the high winds.

Young infections vary considerably in colour; one spot, 28 mm. in diameter, was maize yellow, the centre buckthorn brown, others were chamois with margin and centre of Mikado brown. In a few cases there were one or more concentric rings of brown on a lighter ground. They become much darker with age, varying in colour from Argus brown through light seal brown to blackish brown; frequently the dark central portion is surrounded by a reddish rim.

It is not a soft rot, and the spots are either leathery in texture or else quite hard. A number of affected lemons were kept for some days in a moist chamber and at the end of that time, dirty yellowish drops of a viscid substance were seen oozing from the point of infection. A similar circumstance was observed in lemons artificially infected and kept in a moist chamber.

By cutting through the discoloured spots it can be seen that there is a very definite line between the sound and the diseased tissues, in some cases only about half the thickness of the rind is affected; in others the organism penetrates right through the rind and invades the pulp. The pulp below the discoloured rind becomes rather dry looking and has a peculiar taste and smell; in severe cases it is discoloured to a cinnamon brown, only the outer parts are affected and the disease does not appear to spread in a tangential direction.

On oranges the spots are slightly different in appearance; they average 20—30 mm. in diameter and are not so deeply sunken as those on the lemons; the colour is usually buckthorn brown to Dresden brown; I have never observed spots on oranges with the conspicuous almost black discoloration so common in lemons (Plate VIII).

Naartjes become very badly infected: a number of naartjes sent direct from the orchard shewed as many as 20—30 spots of varying size on a single fruit, they were round to irregular and 1—25 mm. in diameter; slightly sunken and varying in colour from something between Mikado and Sayal brown to blackish brown (Plate VII). In some large spots with an evident thorn prick in the centre the disease

had gone right through the skin and penetrated into the pulp. The latter was not noticeably discoloured but looked rather dry and had a peculiar taste and odour.

On shaddocks by artificial inoculation a number of small spots were produced not exceeding 8 mm. in diameter; they were very slightly sunken, and cinnamon brown rufous in colour. On cutting through these spots it was found that the disease had not penetrated far into the rind; the tissues were discoloured to a dirty brownish yellow to a depth of 1·5 mm., *i.e.*, just below the oil ducts. This discoloured portion was bounded by a thin scarlet line which in some cases marked the boundary between healthy and diseased tissues. The red line is not always present, and when present is not always continuous. The red colouring matter disappeared at once when pieces of the rind were fixed in hot acetic alcohol.

Grape fruit artificially inoculated developed infected areas not unlike those on the lemons. They were sunken, rather irregular in outline, up to 1 cm. diameter, cinnamon colour in the centre with a darker rim, of Mikado or Sayal brown. Sour and sweet limes are also affected in a similar way to the lemons.

On citrons inoculation with a pure culture resulted in the formation of numerous reddened areas up to 12 mm. diameter, some of these were slightly sunken; the colour was at first zinc orange to apricot orange, and later became cinnamon brown.

The only variety of citrus fruit which has so far proved resistant is the Seville orange, which has not been found infected naturally, and which has up to the present resisted infection by artificial means.

Affected fruit cannot be kept for any length of time; the discoloured spots readily serve as a starting point for numerous fungus infections and these complete the work of destruction. Almost every fruit infected with the spot disease, if kept for any length of time, was completely destroyed by *Penicillium spp.* or, less frequently, by *Colletotrichum gloeosporioides.*

The disease not only disfigures and destroys the fruit, but affects the branches to a considerable extent. The organism seems almost invariably to attack the stems just round the leaf bases; an area of about 6—10 mm. diameter becomes water-soaked in appearance, the petiole becomes involved and sometimes the basal part of the leaf, and the leaf falls. The infection then spreads to the branch in the leaf axil, surrounds it at the base and it withers and dries up. The infected area on the main part of the stem becomes up to 20 mm. long, and

goes half way round the stem; after a time the central part loses its water-soaked appearance and turns brown (chestnut brown to olive brown) and becomes slightly sunken (Plate IX, *a* and *b*). The discoloration spreads out to the edges of the infected area which ceases to increase in size. In transverse sections the discoloration does not appear to extend into the wood, but if the bark be peeled off the surface of the woody cylinder appears slightly yellow.

Towards the end of the season, *i.e.*, in the spring, a considerable amount of gummosis may be observed from the affected stem tissues, the gum oozing very largely from the severed leaf bases. In a number of affected branches received at this time of the year the leaf subtending the flowering shoot had been destroyed, and it was a question whether the whole inflorescence would not have dropped before any fruit was set.

If the disease were confined to the fruit it would hardly be expected that it would cause any considerable damage in the districts with a summer rainfall and a dry winter, but the fact of its attacking the branches and causing the destruction of the leaves causes the trees to present a defoliated appearance towards the end of the season. It may be anticipated therefore, that even in a climate where the fruit ripened during the dry season considerable damage would be done to the trees during the summer, and their vitality considerably lowered. An exceptional winter rain such as fell in the Transvaal during last July would be sufficient to start the spread of infection amongst the fruit.

Leaf infections are comparatively rare, occasionally a small discoloured area develops in the leaf tissues, a thorn prick or small wound serving as the starting point of infection. The dark brown spot is surrounded by a yellowish zone and the injured tissues eventually fall out leaving a hole. (Plate IX, *c*).

Inoculation Experiments.

An organism was plated out from lemons received from Simondium in November, 1914; with a pure culture of this a number of lemons were inoculated by needle pricks, and in four to five days these showed a number of small sunken dark-coloured spots. Owing to the pressure of work and the fact that the lemon season was nearly over, the work was discontinued, and was not resumed until July, 1915.

At the beginning of this month the disease again became very active, shortly after the first steady rains in the Western Province, and specimens of infected fruit (both lemons and navel oranges) were

received from the same locality as before. From these a pure culture was readily obtained, and a yellowish bacillus was isolated. With these cultures an infection experiment was carried out; sound lemons obtained from Warmbaths, where the disease does not occur, were dipped in 1:1000 mercuric chloride and then washed in running water. They were then divided into four lots, each lot placed in a large glass jar with a drop-on lid, and treated as follows: in jar (1) the lemons were pricked with a sterile needle; the fruit in (2) was lightly pricked with a very fine needle then atomised with a suspension of an agar culture of the bacillus; in (3) the lemons were inoculated by needle pricks, and in (4) were injected with a hypodermic syringe. At first the lemons were kept at room temperature which even in the day time was only 13° C. After 48 hours there were slight indications of infection, the tissues in the immediate neighbourhood of the needle pricks were slightly discoloured.

After four days infections were quite marked, the oil glands in particular were discoloured, and the tissues in the vicinity of the wound were becoming slightly depressed. The fruit was then removed to the incubator at 25° C. the spots subsequently developed much more rapidly and after ten days all the inoculated fruit showed well developed infections. The fruit in the four jars may be described as follows:

(1) Control, pricked with sterile needle, quite sound, wounds caused by needle pricks barely visible on close inspection. (Plate V, *a*, right hand figure.)

(2) Pricked, then atomised with suspension of culture. There was an infection for almost every needle prick. As many as 100 spots on one lemon, the colour of the spots cinnamon buff to snuff brown and mummy brown; the diseased tissue sunken, and the spots small. (Plate V, *a* and *b*.)

(3) Inoculated by needle pricks, spots up to 6—8 mm. diameter, colour as in (2), from 20—65 sunken spots on each lemon. There was a viscid drop of dirty yellowish substance oozing from the centre of each spot; on examination this proved to be composed of capsuled bacteria, and on being plated out, yielded a pure culture of the organism with which the fruit had been inoculated. (Plate VI, *a*.)

(4) Suspension of culture in distilled water injected with hypodermic syringe; spots varied from 3 mm. to 30 mm. diameter; they were sunk 1—1½ mm. below the surface, 4—32 spots on each fruit, some of which did not appear to be in connection with a wound. The discoloration varied from Sayal brown to mummy brown. Viscid

drops of bacterial slime were also oozing from the centre of these spots. (Plate VI, *b*.)

An infection experiment on a larger scale was carried out with a quantity of citrus fruit obtained from the Government Orchard at Warmbaths; the fruit was picked from the trees in the writer's presence, taken direct to the Laboratory in boxes and the inoculations carried out two days later. There was no trace of the disease on any of the fruit in the orchard.

The cultures used were from four different sources: from natural infections on lemon, on orange and on naartje and from an artificial infection on lemon. In the schedule the last is distinguished as lemon II, the first as lemon I. In each case young cultures on nutrient agar were employed for needle prick inoculations, and young cultures suspended in sterile distilled water for atomising.

All controls were pricked with a sterile needle; inoculations were carried out in four different ways, some were atomised with a "glaseptic" sprayer without any previous wounding, others were lightly pricked with a very fine steel needle and then atomised, into a third lot of fruit the organism was introduced by means of needle pricks, and the bacillus was injected into a fourth set by means of a hypodermic syringe.

No. of Fruit	Kind of Fruit	Source of Culture	Method of Inoculation	Result
6	Ripe lemons	—	Sterile needle	No infection
7	,, ,, ...	Lemon I	Pricked then atomised	Positive
8	Green ,, ...	,, I	,, ,, ,, ...	,,
7	Ripe ,, ...	,, I	Needle pricks	,,
8	Green ,, ...	,, I	,, ,, ...	,,
7	Ripe ,, ...	,, I	Hypodermic syringe ...	,,
7	Green ,, ...	,, I	,, ,, ...	,,
5	Ripe ,, ...	—	Sterile needle	No infection
5	Ripe ,, ...	Orange	Pricked then atomised	Positive
5	Green ,, ...	,,	,, ,, ,,	,,
6	Ripe ,, ...	,,	Needle pricks	,,
5	Green ,, ...	,,	,, ,, ...	,,
7	Ripe ,, ...	,,	Hypodermic syringe ...	,,
7	Green ,, ...	,,	,, ,, ...	,,
5	Navel oranges	—	Sterile needle	No infection
5	,, ,, ...	Lemon I	Pricked then atomised	Positive
6	,, ,, ...	Orange I	,, ,, ,,	,,
5	,, ,, ...	Lemon I	Needle pricks	,,
5	,, ,, ...	Orange	,, ,, ...	,,
5	,, ,, ...	Lemon I	Hypodermic syringe ...	,,
5	,, ,, ...	Orange	,, ,, ...	,,
8	Valencia late oranges	—	Sterile needle	No infection

No. of Fruit	Kind of Fruit	Source of Culture	Method of Inoculation	Result
15	Valencia late oranges	Lemon I	Needle pricks	Positive
8	,, ,, ,, ...	Orange I	,, ,, ...	,,
10	Seedling oranges (Parson Brown)	—	Sterile needle	No infection
11	,, ,, ,, ,,	Lemon I	Needle pricks	Positive
10	,, ,, ,, ,,	Orange	,, ,, ...	,,
3	Seville oranges	-	Sterile needle	No infection
3	,, ,, ...	Orange	Needle pricks	Doubtful
4	,, ,, ...	Lemon I	,, ,, ...	,,
3	,, ,, ...	Orange	Pricked then atomised	,,
1	Citron	—	Sterile needle	No infection
1	,, ...	Lemon II	Needle pricks	Positive
1	,, ...	Orange	,, ,, ...	,,
1	,, ...	Lemon II	Pricked then atomised	,,
1	,, ...	,, II	Hypodermic syringe ...	,,
8	Naartjes	,, II	Atomised without wound	Negative
4	Navel oranges	Orange	,, ,, ,,	,,
4	Sweet limes	Lemon II	,, ,, ,,	,,
4	Naartjes	—	Sterile needle	No infection
5	,, ...	Lemon II	Needle pricks	Positive
5	,, ...	Orange	,, ,, ...	,,
6	Sour limes		Sterile needle	No infection
6	,, ,, ...	Lemon II	Needle pricks	Positive
6	,, ,, ...	Orange	,, ,, ...	,,
7	,, ,, ...	Lemon II	Pricked then atomised	,,
5	Navelencia oranges	,, II	Atomised without wound	Negative
4	Sweet limes	,, II	Needle pricks	Positive
4	,, ,, ...	Orange	,, ,, ...	,,
5	,, ,, ...	Lemon II	Pricked then atomised	,,
4	,, ,, ...	Orange	,, ,, ,,	,,
5	,, ,, ...	Lemon II	Hypodermic syringe ...	,,
7	Sour limes	—	Sterile needle	No infection
5	,, ,, ...	Lemon II	Atomised without wound	Negative
5	,, ,, ...	Orange	,, ,, ,,	,,
5	,, ,, ...	Lemon II	Hypodermic syringe ...	Positive
12	Lemons	,, II	Atomised without wound	Negative
1	Shaddock	—	Sterile needle	No infection
1	,, ...	Lemon II	Needle pricks	Positive
1	,, ...	Orange	,, ,, ...	,,
4	Grape fruit	—	Sterile needle	No infection
4	,, ,, ...	Lemon II	Needle pricks	Positive
4	,, ,, ...	Orange	,, ,, ...	,,
1	,, ,, ...	Lemon II	Hypodermic syringe ...	,,
1	,, ,, ...	Orange	,, ,, ...	,,
16	Rough lemons	Naartje	Needle pricks	,,

It will be observed from the above schedule that with the exception of the Seville oranges all inoculations were positive on fruit which had

been pricked or otherwise slightly wounded. None of the lemons or other citrus fruit which were atomised without wounding developed any spots, but I do not regard this as conclusive evidence that the organism cannot find its way through the stomata, for several reasons. In the first place, the fruit which was used for the experiment had been hanging on the trees in a very dry atmosphere, and the outer tissues of the rind had become comparatively dry and leathery, and it was noticed that even after wounding these infected less readily than those used in the preliminary experiment which were cut after an exceptional spell of rainy weather; also in the first experiment a number of infections appeared on lemons which were injected with a hypodermic syringe at points where no injections had been made, and on naturally infected fruits on many spots where no wound can be found, and sections through the tissues show the bacteria in the substomatal cavity, but this will be alluded to later.

Further inoculations will be necessary before anything conclusive can be arrived at with regard to the possibility of stomatal infection, but obviously the most frequent method of infection is through wounds.

On the trees the spots are only found on ripe fruit, and two or three farmers have reported it as occurring only on fallen fruit or developing in the store room. The organism is very probably a soil bacillus which first invaded fruit lying on the ground and has now taken on a parasitic habit. It was found quite possible to infect green lemons, although the organism attacked them less readily than the ripe fruit.

The organism loses its virulence rather rapidly on artificial media; this was clearly shown by inoculating two similar sets of lemons, one with a freshly isolated culture, and the other with a culture isolated about a month previously and transferred fifteen times. On the first, signs of infection were visible after 48 hours and distinct spots in four days; on the second, the discoloration was much delayed; it was not visible till the fourth or fifth day and the infected areas comparatively small and poorly developed.

The identity of the organism in fruit and branch was also tested. A culture of this bacillus isolated from a leaf base infection was used to inoculate a number of sound lemons with positive results.

A strain isolated from lemons sent from the Constantia Wine Farm produced a few positive infections on some young trees growing in tins in the Laboratory grounds. From these the organism was again isolated and employed with positive results to infect a number of lemons.

Pathological Histology.

The organism was most easily studied in the rind of affected fruit; satisfactory preparations were obtained by fixing in hot acetic alcohol and staining with Ziehl's carbol fuchsin and light green.

This is essentially a disease of the parenchyma, the lignified tissues of the stem are not invaded, and the organism is not found in the small fibro-vascular bundles in the rind. The bacillus does not cause hyperplasia.

As has been stated, in the majority of cases infection takes place through a wound; the very slightest abrasion with a fine needle being sufficient. No definite evidence has yet been obtained by inoculation, that stomatal infections are possible, but in a number of sections, particularly those through incipient spots on naartje rind, the location of the bacilli strongly suggested that they had effected an entrance through the stoma. (Plate X, *a*.) In such cases the stomatal cavity was occupied by a mass of bacteria and the surrounding intercellular spaces had also been invaded. The rods multiply very rapidly in the intercellular spaces, until the latter become much distended and bulge into the cell cavity. Considerable tension is thus set up and eventually the wall gives way and the bacilli force an entrance into the cell.

The cell cavity soon becomes entirely filled, but for a considerable time the remaining walls are intact so that were it not for the absence of any intercellular spaces it would appear that the organism was intracellular. The cell contents become disorganised and disappear with the exception of the protoplasmic lining of the cell wall which contracts slightly away from the wall and stains deeply with fuchsin. The cells at this stage consist of a central mass of bacteria surrounded by a deeply stained sack of disorganised cell contents and then by the partially destroyed cell wall. The staining reaction of the latter is not altered. Finally the intervening walls give way and the tissues become completely disorganised. (Plate X, *c*.)

The oil gland is often completely filled with a vast number of rods; it was afterwards found that the organism grows extremely well in the presence of orange oil and lemon oil. The cells surrounding the oil glands are also very frequently attacked.

Morphology.

The organism is a rather slender rod with rounded ends; the living rods diffusing from discoloured cells in the rind of a lemon measured ·8 to 3·2 μ by ·5 to ·7 μ; in a smear from the same source stained with dilute fuchsin they measured 1·4 μ by ·45 to ·7 μ, the majority in each case were about 1·5 × ·5 to ·6 μ. Similar measurements were recorded for rods from diseased tissues of spots on oranges and naartjes. The bacilli in the viscid mass oozing from affected fruit are slightly stouter on the whole than those in the tissues, most of them are ·6 to ·65 μ in thickness, in length there is not much variation.

Measurements of rods from a 24 hour culture on nutrient agar and in nutrient broth coincided with those of rods taken direct from the tissues of the host plant, but on gelatine they are considerably shorter, the majority measuring ·8 to 1 μ by ·5 to ·6 μ. In Uschinsky's solution short forms also predominate.

In old agar streaks the rods vary very much in length, some are almost spherical, others attain a length of 5 μ before dividing; some stain intensely, others faintly or unevenly.

No long filaments have been observed in the pellicle on sugar broth, the longest unsegmented forms noticed in these media were about 8 μ in length.

The sediment in three months old broth cultures consists for the most part of rather short rods, some of which stain intensely with gentian violet, but the majority stain faintly or unevenly. In old cultures on steamed potato there are also a large number of individuals which only stain faintly, and these are considerably swollen so as to be ellipsoid or almost spherical in form.

Fission. The multiplication of the organism by fission was studied on the agar hanging block; a smear was made from a 24 hour old broth culture which had been incubated at a temperature of 35° C., the preparation was illuminated by a Nernst lamp, and the lenses used were a Zeiss $\frac{1}{12}''$ oil immersion objective and No. 12 compensating ocular. With this magnification the alteration of the rods in size and form could be easily followed, and drawings were made with the camera lucida at frequent intervals. The temperature of the room was approximately 26° C.

At 9.15 a.m. a single rod was selected for study (Plate XII, *a*). It at once elongated somewhat, became constricted and by 10 a.m. had divided into subequal rods. The upper of the two new individuals

thus formed had divided again by 10.45, the lower became constricted and divided soon after. Subsequently each rod took approximately 20 minutes after separation to attain to its maximum size, and then immediately became constricted and fission was complete in another 20 minutes. Each individual became slightly curved or bent when it became constricted previous to fission, and when division was completed the resulting rods were not exactly equal. The newly formed individuals at once became separated by an appreciable gap, their close contact being prevented by the capsule which could be detected by a careful adjustment of the illumination.

At 1 p.m. the young colony consisted of 16 rods; by 1.30 the number had increased to 22 and it was no longer possible to make accurate drawings as many of the rods had oriented themselves with their long axes parallel to the line of vision and they were overlapping in various directions.

No further observations were made until 9.15 on the following morning; at that time the surface of the block was covered with highly refractive moruloid masses of bacteria; the capsuled bacteria in each mass were surrounded by a common envelope with a high refractive index, and outside of this were a large number of rods lying free (Plate XII, *b*). When first examined the free rods round the masses near the edge of the block which were probably better supplied with oxygen than the rest were in active motion, and large numbers of them had made their way from the agar to the surface of the cover glass beyond which was covered with a thin film of moisture. After about 15 minutes' exposure to brilliant illumination from the Nernst lamp all these rods came to rest.

Grouping. The rods are most commonly single or in pairs; loose chains are found in the pellicle and also in sediment in some sugar broths, particularly on nutrient broth with 2 per cent. laevulose, but these do not as a rule consist of more than 20 or 30 elements (Plate XIII, *a*) and they lie amongst large numbers of single rods. There are no chains in the ring above the liquid.

On solid media the organism almost invariably forms itself to a greater or less extent into the capsuled, moruloid masses described above, they are also found in the pellicle on ordinary nutrient broth. If such masses be mounted in water they do not resolve themselves into chains but isolated rods disentangle themselves and swim away in a state of high activity.

Motility. The organism is often sluggishly motile when it is taken

direct from the tissues of the host, and if a small portion of the viscid mass oozing from affected areas on the fruit be mounted in water the rods diffuse out and immediately become very active.

It is extremely active in young cultures; an examination under the low power of the microscope of 18 to 24 hour old colonies on nutrient agar shows the outer part of each colony as a swirling mass of actively motile rods. In liquid media the bacillus retains its activity for a considerable length of time; chains from the pellicle of a 16 days old broth culture separated and immediately became very active on being transferred to water.

The motility of the organism can be very well studied in preparations made from a young agar culture and observed with the dark ground illumination. The rods move forward with a steady sinuous motion which is frequently interrupted by rapid oscillating movements.

The flagella stain quite readily; in the living state they may be stained by Straus' method (1). The position of the flagella can be well made out, they are obviously peritrichous but are in too rapid motion for any accurate estimate of their number. With Ellis' modification of Loeffler's method very satisfactory preparations were obtained. There are 5 to 10 long peritrichous flagella: these are several times the length of the rod, measuring roughly 8 to 12 μ. They are not distributed evenly over the surface but frequently consist of one or two groups of 3 to 5 which are crowded up towards the poles (Plate XII, *c*).

Capsules. The viscidity of the substance oozing from diseased tissues and the leathery or mucilaginous texture of many cultures on solid media at once suggest the presence of a capsule.

A capsule may almost always be detected and may be stained either by one of the special capsule stains, by a dilute solution of fuchsin or with carbol gentian violet. The viscid drops oozing from the fruit consist of capsuled rods embedded in a tenuous slime which appears granular when stained by MacConkey's method (Plate XIII, *d*).

In an impression preparation of a young agar colony all the rods were definitely capsuled (Plate XIII, *c*) and in the older parts of the colony become crowded together into small tough masses which are firmly embedded in the medium. In certain cases streak cultures on agar take on a roughened or shagreen-like appearance, and the streak becomes leathery and can be torn in a strip from the surface of the medium. Old streaks of raised, shining, more homogeneous appearance are usually mucilaginous; the difference in texture appears to have some relation to the amount of water present in the medium.

No capsules have been observed around rods within the tissues of the host.

The capsule around each rod in an impression preparation stained with fuchsin measures roughly 1·5 μ in diameter and it exceeds the rod in length by about ·5 μ at each end. A few rods may be seen without capsules and empty capsules are quite common, but it was not clear whether the rods escape from the capsules or whether they had been torn away in making the preparation.

Involution forms were observed in broth cultures containing high percentages of sodium chloride. They are very common in 48 hour old cultures in broth containing 9·5 per cent. to 10·5 per cent. NaCl. The rods comparatively are very large, some grow out into filaments as much as 100 μ long, and they are irregularly swollen and contorted in every direction (Plate XIII, *e*). Quite a number are club-shaped. They stain intensely with aniline gentian violet and when transferred to ordinary nutrient broth rapidly divide and produce rods of normal form.

Aberrant forms are also found in old potato cultures. A smear made from a 12 days old potato culture consists of very minute intensely staining capsuled forms, some of which are almost spherical and slightly swollen rods of normal length which stain very feebly or irregularly. The latter were only faintly stained in half-an-hour by carbol gentian violet (Plate XIII, *b*).

Staining reactions. The bacillus stains well by all the ordinary methods, but varies considerably in its reaction to the various dyes. In testing the action of the different stains smears were made from a 24 hour old streak on nutrient agar. Dilute aqueous solutions were allowed to act for two minutes; in this time the thionine and methylene blue hardly stained the rods at all, fuchsin gave a distinct though rather faint coloration and the rods stained with gentian violet were very intensely and quite sharply stained. Carbol gentian violet also gave a very strong intense stain in half a minute and brought out the capsules which were not stained by the aqueous solution. Ziehl's carbol fuchsin gave a similar result; carbol methylene blue and carbol thionine also stained very faintly in half a minute, but Loeffler's blue gave much better results in the same time though not nearly so intense a stain as the gentian violet and fuchsin.

The organism is Gram-positive, and stains blue by the Ziehl Neelson method, *i.e.*, it is not acid fast.

Spores. No spores have been observed.

Cultural Characters.

Nutrient agar colonies. The organism grows very rapidly on nutrient agar and when first isolated from the tissues the speed with which the colonies appear varies considerably; after preliminary cultivation however, more even results are obtained. The following is a description of the development of typical colonies at 25° C.

After 24 hours in thinly sown plates the colonies are 1—4 mm. in diameter, they average 2 mm., and are round-irregular in shape; by transmitted light translucent with a small opaque centre, pearly white to bluish, yellowish by reflected light. Under the microscope the colony is granular with a grumose centre, the granular margin being composed of a swirling mass of actively motile rods. Submerged colonies are minute opaque, mostly bi-convex.

After two days the average diameter of the colonies has increased to 4 mm., and some measure as much as 7 mm., they are coppery by transmitted light with a bluish margin, straw yellow by reflected light. There is a small opaque centre surrounded by a rather dense sub-opaque ring and then by a translucent margin. Under the low power of the microscope the central portion is grumose, the outer finely granular.

Submerged colonies have increased somewhat in size and now have the appearance of two or more lenticular bodies placed at different angles, or of a single lenticular body with sub-spherical outgrowths.

After three days the colonies average 5—6 mm. in diameter, the largest are 10 mm., they are round-irregular, umbonate; Naples yellow to buff yellow by reflected light, coppery throughout by transmitted light; under the microscope they are much more dense than before except at the edge, and the whole of the central part is grumose; there is no longer any motion visible with a low magnification. Submerged colonies have increased in size up to $\frac{1}{2}$ mm.

After four days the colour has become mustard yellow and long feathery crystals have made their appearance in the medium.

There is usually no further change after the fifth day; the colonies then measure up to 15 mm. in diameter, the margin is entire or undulate, and there are frequently two or three concentric rings. The surface of the agar has become distinctly whitened especially in the immediate vicinity of the colonies.

The growth in this case does not become tough or leathery, it is always creamy in consistency.

The size of the colonies is very much affected by crowding. (Plate XIII, *b, c, d.*)

Nutrient agar streak. A well developed agar streak appears in 24 hours at 25° C.: it is smooth, shining, about 3 mm. wide and grumose in the centre. In three days this has become 3—5 mm. broad, primuline yellow, the central part being pulvinate, and the margin entire or slightly undulate.

After 12 days the surface of the agar has become very decidedly whitened except for the parts covered by the bacterial growth; long feathery crystals point downwards into the medium from the under surface of the streak. The condensation water is very heavily clouded. A second kind of crystal is formed on the surface of the agar under the condensation water, very similar to those illustrated by Smith as occurring in cultures of the olive tubercle organism (7).

The texture of the streaks in three weeks old culture varies very much with the amount of water present in the medium and in the atmosphere. Some remain creamy, but contain small tough moruloid masses; others become very tough at an early stage, large strips may be pulled away on the platinum needle and when this is done the growth stretches somewhat and has the appearance of a number of spherical colonies in a tough homogeneous matrix. Most commonly the growth is raised and almost homogeneous to the naked eye. It is then very mucilaginous, and in colour and texture is not unlike plastic sulphur.

If the surface of the sloped agar be bathed in lemon or orange oil a very luxuriant growth is obtained which does not become tough or mucilaginous.

Nutrient agar stab, the best growth is at the surface.

Glucose formate agar. Streaks were made on this medium as a control for those kept under anaerobic conditions; the growth is very similar to that on ordinary nutrient agar, but slightly more luxuriant.

Sulphindigotate agar. This medium was used for a similar purpose; a very luxuriant growth was obtained, on the upper drier portion of the slope a number of small discrete colonies were produced which at first absorbed the colouring matter from the medium and became blue. The lower part of the slope was almost covered by a wet shining, yellowish growth which was grumose in the centre. The colour gradually disappeared from the medium.

Lemon and *Orange glucose agar.* Colonies on these media are similar to those on nutrient agar but considerably smaller, only 2·3 mm. in diameter after three days; they are very markedly umbonate in

the centre, and concentric rings are visible by transmitted light; under the low power of the microscope they are grumose in the centre and coarsely granular near the edge; in colour they are dirty yellowish white. After five days the diameter has increased to 5 mm., and the colonies have a number of distinct concentric zones, by transmitted light the two central zones are yellow and the remainder bluish white.

Lemon and orange glucose agar streaks[1] are dirty yellowish white, glistening, very slightly raised, 3—10 mm. broad, and the condensation water is rather heavily clouded. On the lemon glucose agar the streak had a shagreen surface, on orange glucose agar the surface was smooth but moruloid in the centre.

Orange laevulose agar. Streaks on this agar are similar in character to those on orange glucose agar but are considerably more luxuriant.

Beerwort agar. The organism grows very well on this medium, it forms a shining streak with a convex central portion and a flat margin with a slightly undulate edge; the breadth of the central portion is 4—5 mm., of the margin 1 mm. There is a copious yellow growth in the condensation water.

As it dried up the surface of the raised central part becomes corrugated and it is decidedly viscous.

Bean agar is a fairly good medium, streaks are smooth, yellowish, somewhat umbilicate, and about 3 mm. diameter.

Cabbage agar. A very characteristic growth forms on streaks of cabbage agar. It is not very abundant and consists of a number of spherical, opaque, yellowish colonies standing up above the surface of the medium, and disposed irregularly in a flat translucent streak 1—4 mm. wide. The surface of the agar becomes conspicuously whitened in the vicinity of the streak.

Loeffler's blood serum is not liquefied, the organism forms a shining yellow growth along the needle track.

Starch jelly. No growth.

Nutrient gelatine colonies are just visible to the naked eye after 24 hours at 20° C. as minute glistening points. In two days they attain a diameter of 1—3 mm., and are round, glistening, slightly convex, coppery by transmitted light, creamy by reflected light. Under the microscope they are rather coarsely granular and inclined to become

[1] *Orange or Lemon glucose agar* was made as follows: 50 grammes of fresh rind was boiled for 30 minutes with 500 c.c. distilled water, filtered, then 10 grs. dextrose added and solidified in the usual way with 1·5 agar powder.

In *Orange laevulose agar*, laevulose was substituted for *glucose*.

grumose in the centre. Submerged colonies are minute, white and subspherical.

Seven days old colonies are up to 4 mm. diameter, apricot yellow, they are beginning to sink in the centre owing to the softening of the gelatine, the growth is slightly viscous. In nine days the gelatine in the immediate vicinity of the colonies is liquefied, and in 14 days liquefaction is complete. The colonies are still almost intact and floating in the liquefied medium.

Nutrient gelatine shake cultures develop very numerous minute colonies, the majority of which are within 1 cm. of the surface of the medium, which is slowly liquefied.

Nutrient gelatine stab. The organism grows comparatively slowly at 20° C., the growth along the needle track being barely visible at the end of 24 hours. After seven days there is a fairly thick line of growth all along the needle track, the surface colony is slightly sunken in a small crater of liquefied gelatine. In 14 days liquefaction is napiform, the surface growth sinks to the bottom of the liquefied gelatine, continually re-forming and sinking, thus forming a considerable amount of sediment. The liquefied portion is clouded, and there are very numerous minute opaque yellowish flocculi in suspension throughout it. (Plate XI, *a*.)

After 30 days the liquefaction is broadly saccate and extending to the bottom of the tube, and in 6—7 weeks the gelatine is completely liquefied.

Nutrient gelatine streak. In three days there is a shining yellowish streak about 2 mm. wide and slightly raised in the centre. Liquefaction begins on the fourth day; a small pocket of gelatine liquefying near the bottom of the streak. The following day there is a groove along the surface of the slope, from which the liquefied gelatine has run carrying the yellowish growth to the bottom of the tube. A fresh streak forms in this groove, in its turn to be carried to the bottom of the tube, and this process is repeated several times. The yellow growth does not diffuse readily into the liquefied medium.

Potato. On potato the bacillus grows well, a thin spreading yellowish growth almost covers the sloping face of the cylinder in 24 hours at 25° C.; after three days the growth is primuline yellow to mustard yellow, glistening, spreading, covering all the lower part of the cylinder except where it is in contact with the tube and the greater part of the upper drier portions. No growth is visible in the liquid in the bulb. The potato is distinctly browned, the discoloration being most marked in the tubes containing glycerinated potato.

Carrot. The water in the bulb of the Roux' tube is distinctly clouded, on the carrot there is a plentiful, colourless wet-looking, shining growth spreading over the medium.

Turnip. Growth is much less abundant than on carrot, it is not quite colourless but has a yellowish tinge.

Beet. Here the growth is very similar to that on carrot but is distinctly yellow, and there is a yellowish ring at the surface of the liquid in the bulb of the Roux tube.

Orange and lemon rind steamed show after three days glistening, dirty yellowish masses of slightly viscid bacilli raised above the surface of the rind.

Nutrient bouillon (+ 15 Fuller). Nutrient broth clouds in six hours at temperatures from 25° to 35° C., and in 24 hours there is a distinct suggestion of pellicle formation. The pellicle is thin and falls to the bottom of the tube if the latter is shaken slightly. Three months old cultures are almost clear; there is a small amount of dried up growth on the tube where the surface of the medium originally was and a considerable amount of deposit. The rods in the deposit are living and multiply readily when transferred to fresh tubes of broth.

Pellicle formation takes place most readily on broth with an acid reaction, on alkaline broth little or no pellicle is formed. After standing for some days flasks of acid broth showed a thin pellicle in which were suspended numerous more opaque portions which are shining, yellowish and rather greasy looking. In some lights the pellicle is distinctly iridescent.

The ring above the liquid does not become tough or mucilaginous, and the pellicle is flocculent.

Sugar bouillons. The growth in dextrose broth is not particularly good; a heavier clouding was obtained in broth containing maltose, lactose and galactose, and the best growth of all in 2 per cent. laevulose broth.

Litmus milk. In milk the bacillus grows comparatively slowly; no change could be observed for seven days, after that period the milk became slightly more acid in reaction and the litmus was then gradually reduced. After 10 days at 35° C. the casein coagulated and there was a gradual extrusion of whey. The whey was not abundant, almost clear, and yellowish in colour, a sediment of yellow bacteria gradually accumulated on the surface of the coagulum; the latter did not become peptonised.

Milk serum, filtered through a porcelain candle, proved to be

a favourable medium. The liquid became turbid and a decided whitish pellicle formed on the surface.

Dunham's solution became only very slightly clouded; no pellicle and very little sediment.

Cohn's solution. No growth.

Uschinsky's solution at first is only slightly clouded but a thin tough pellicle forms; this increases in size, remaining adherent to the sides of the tube and becoming saccate and dipping below the surface of the solution in the centre of the tube. This pellicle finally sinks to the bottom and the liquid then becomes densely clouded and baryta yellow to buff yellow in shade. There is a decided ring above the liquid which becomes ochraceous tawny to cinnamon brown.

Cabbage broth. There is not much clouding and only a small amount of sediment. The pellicle however is quite conspicuous, it consists of vast numbers of minute, opaque, yellowish bodies and presents a frosted appearance.

Lemon juice filtered through a porcelain candle and undiluted proved to be too acid a medium for growth.

Nitrate broth is heavily clouded but no pellicle forms owing to the vigorous evolution of gas. The broth is covered with froth to the depth of 1 cm., for some days.

Physical and Biochemical Features.

Enzyme production.

Proteolytic enzymes. Milk serum cultures tested at the end of five days gave only negative results; after 10 days however, although the culture gave negative reactions for proteose and peptone there was a faint but unmistakable reaction for tyrosin which was absent in tests made from the control flask.

Diastase. Potato cylinders on which the organism had been grown gave a distinct red brown reaction with iodine indicating the presence of amylodextrin. The same reaction was observed in peptone water containing potato starch, but in neither case was there any reaction for sugar with Fehling's solution.

Nutrient bouillon cultures to which were added 2 per cent. thymo and equal quantities of starch paste did not precipitate Fehling's solution after six to eight hours.

Invertase was also absent from broth cultures.

Acid production. In nutrient broth containing 2 per cent. dextrose, laevulose, saccharose, lactose and glycerine and in ordinary broth without the addition of any carbohydrate there is no acid production, but the medium in 10 days becomes slightly more alkaline, but only by 10 degrees or less of Fuller's scale.

In peptone water tinted with litmus, however, the organism produced acid with several carbohydrates. The following table shows the behaviour of the organism in tubes containing Dunham's solution and various carbohydrates and other substances.

Dextrose	Slightly acid, litmus somewhat reduced
Laevulose	Acid reaction marked
Galactose	Slightly acid, litmus considerably reduced
Maltose	Distinctly acid
Lactose	No change in reaction
Saccharose	Slightly acid, litmus somewhat reduced
Dextrin	No change in reaction, litmus completely reduced
Starch	No change in reaction
Glycerine	No change in reaction
Mannite	Distinctly acid
Sodium formate	No change in reaction
Sodium citrate	No change in reaction

The bacillus therefore produces acid in the presence of laevulose, dextrose, galactose, maltose, saccharose and mannite, most marked with laevulose.

Alcohol production. The first distillate from a culture in 2 per cent. laevulose broth was tested for alcohol, etc. The distillate was divided into four portions, to the first was added Lugol's iodine, then a little NaOH solution; on heating the mixture there was a distinct smell of iodoform. The second portion tested with Schiff's reagent gave a negative reaction for aldehyde. To 10 c.c. of a third portion was added 2·5 c.c. 25 per cent. sulphuric acid and a crystal of potassium bichromate. This was distilled, and in the process the bichromate was reduced to a green colour, and the distillate smelled strongly of acetaldehyde and reacted with Schiff's reagent.

A similar test with distillate from control flask gave only negative results. It is evident therefore that an appreciable amount of alcohol is produced in sugar broth cultures.

Ammonia production. The distillate from cultures in nutrient broth gave a positive reaction for ammonia with Nessler's solution.

Indol is produced both in Dunham's solution and in nutrient broth incubated for 10 days at 25° C., but all tests for *Phenol* were negative.

Pigment production has been described in various media in connection with the cultural characters of the organism. The pigment is insoluble in water (hot and cold), alcohol, ether, chloroform, carbon bisulphide and dilute acids and alkalis.

Reducing agent formation.

Colour reduction. The reducing power of the bacillus is very slight; it was tested in nutrient broth tinted with various coloured solutions. Rosolic acid and indigo carmine are not reduced. Methylene blue becomes peacock green in two days and in five days is completely reduced. Neutral red which was at first flame scarlet in four days was reduced to orange buff but was completely reduced only at the end of 15 days.

Litmus showed no sign of reduction until after 15—21 days when there was a small amount of colourless liquid at the bottom of the tube, after a few days the colour again became even.

Reduction of nitrates. In nitrate broth the nitrates are reduced with evolution of gas, the evolution being most vigorous at 25° C. from the second to the fourth day, on the fifth day it ceased. The tubes were tested at the end of the tenth day and gave no reaction for nitrate or ammonia.

In nitrate peptone water no gas is evolved and after 10 days the solution gives positive reactions for both nitrates and ammonia.

In control tubes both tests were negative.

Gas production. No gas is evolved in fermentation tubes containing bouillon or peptone water and any of the substances mentioned in connection with acid production. The organism grows in the closed end of the fermentation tube but frequently the clouding is only slight. In iron peptone and lead peptone solution there was a distinct blackening of the precipitate as compared with the control tubes; a small amount of sulphuretted hydrogen is therefore present.

Atmosphere. The organism is a facultative anaerobe, as suggested by its ability to grow in the closed end of the fermentation tubes.

Four sets of cultivations were prepared, one of which was sealed in Buchner's tubes containing an alkaline solution of pyrogallic acid, and the other three were each placed in a Bulloch's apparatus.

From the first the air was exhausted as completely as possible so that the organism was growing under reduced pressure; from the second the oxygen was absorbed by pyrogallic acid, and the air in the third was displaced by CO_2.

The bacillus grew almost as luxuriantly under reduced pressure as in the control tubes. In the absence of oxygen, *i.e.*, practically in an atmosphere of nitrogen, and in CO_2 growth was comparatively very slow and restrained.

Temperature. The bacillus grows through a wide range of temperature, and very rapid multiplication takes place at all temperatures from 25° C. to 38° C. The optimum temperature is 35° C. At 15—18° C. the organism grows very slowly, and at 0° C. no clouding can be observed in tubes of nutrient broth. The organism is not killed by long exposures to this temperature, but rapidly clouded tubes transferred to a warmer temperature after 10 days. The upper limit for growth is 43° C., broth is not clouded at 45° C. and the bacillus is destroyed by several days exposure to this temperature.

The thermal death point (in standard 10 c.c. tubes 10 minutes exposure) is 62° C.; dry on cover slips it is 110° C.

Reaction of medium. The organism is not very sensitive to the reaction of the medium and grows almost equally well in broth with reactions of + 15 to + 25, the optimum however is about + 20 of Fuller's scale. It grows through a wide range and can grow in a medium of fairly high reaction if the acid used is malic, citric or tartaric.

Substance tested	Maximum of growth	Amount to inhibit growth
Acetic acid	+25	+ 30
Oxalic acid	+25	+ 30
Citric acid	+95	+100
Tartaric acid	+95	+100
Malic acid	+95	+100
Sodium hydrate	−40	− 45

Toleration of NaCl. The percentage of salt which the organism can tolerate is surprisingly high; some slight growth took place in tubes containing 10·5 per cent. NaCl and those with 10 per cent. were decidedly clouded. Involution forms are however produced in broth containing high percentages.

Desiccation. A young agar culture was suspended in water and smeared on cover slips. As soon as the smear was dry, these were transferred to a desiccator; they were removed from time to time and dropped into tubes of nutrient broth. The organism was still alive after 80 days, more prolonged tests have not yet been carried out.

Sunlight. The test was carried out in very bright sunlight soon after midday in the month of October. Thinly sown plates were

employed, one half being covered with black paper. To prevent the effects of a rise in temperature, each plate was covered with a small glass dish filled to a depth of 2 cm. with a 2 per cent. solution of potash alum. The organism was killed by an exposure of 60 minutes and the number considerably reduced in 45 minutes.

Chloroform. The bacillus will not grow in nutrient bouillon over chloroform.

Germicides. The following table will serve to indicate the behaviour of the organism toward the more common germicides:

Substance	Maximum for growth	Amount to inhibit growth	Amount to kill organism in 30 minutes
Copper sulphate	1 : 10000	1 : 5000	1 : 1500
Carbolic acid	1 : 1000	1 : 500	1 : 100
Mercuric chloride	1 : 10000	1 : 5000	1 : 4000
Formalin	1 : 1000	1 : 750	1 : 50

It will be observed from the above schedule that the organism is very sensitive to copper sulphate, it is to be hoped therefore that good results may be obtained by spraying the infected trees with Bordeaux mixture.

PATHOGENICITY TO ANIMALS.

Through the courtesy of Sir Arnold Theiler, the pathogenicity of the organism to animals was tested at the Veterinary Research Laboratories at Onderstepoort. I am indebted to Mr E. M. Robinson for the following notes on experiments which he has carried out in this connection.

To test the pathogenicity of the Bacillus of Lemon disease, two rabbits were inoculated each with half an agar slope of a 24 hours' growth of the organism. One rabbit which will be called (A) was inoculated on 25th September, 1915, and the inoculation was given intraperitoneally. This rabbit developed symptoms of extreme illness and on the two days following the inoculation would not eat, refused to move and occasionally made grinding movements with its teeth. On the third day after the inoculation the rabbit appeared much less dull and at the end of a week appeared quite well again. On the 7th October, 1915, the rabbit received a further inoculation of half an agar slope (24 hours' growth) subcutaneously, but showed no further symptoms of illness, and is still healthy to date (14th November, 1915).

Another rabbit (B) was inoculated with half an agar slope of the organism (24 hours' growth) subcutaneously on the 25th September, 1915.

It developed no symptoms of illness and on 7th October, 1915, received a further similar subcutaneous inoculation, the same dose being used. On 11th October the rabbit showed symptoms of severe illness with thick mucopurulent discharge from the eyes and extreme dullness. On 12th October, 1915, it was found dead, and the post mortem examination showed nothing but a slight hyperaemia of the lungs. Cultures from the heart's blood remained sterile. This rabbit's death was probably not due to the bacillus of Lemon disease as the inoculation of the first rabbit (A) was a much more severe test of pathogenicity.

Intravenous inoculations were not attempted as it has been found that most non-pathogenic organisms will produce death from toxaemia except in very small doses.

Rabbit (A) was bled on 12th November, 1915, and its serum tested for agglutinins against the organism. Agglutination was obtained in a dilution of 1—800 distinct, and partial in a dilution of 1—2000. The serum of a normal rabbit gave no agglutination in as low a dilution as 1—10. Fresh orange juice would not agglutinate the bacillus in any dilution.

The agglutination test should prove of use in the ultimate proof of organisms causing plant diseases and is quite easy to apply.

Comparative table of characters of three organisms causing diseases of Citrus.

	Bacillus citrimaculans	Bacterium citriputeale	Pseudomonas citri
Nature of disease produced	Dark sunken spots	Dark sunken spots	Canker
Organs affected	Fruit, stems, rarely leaves	Fruit	Fruit, stems and leaves
Form of organism	Rod with rounded ends	Rod	Rod with rounded ends
Dimensions	$1—4 \times \cdot 45—\cdot 7\mu$	$2—4 \times \cdot 51—1\mu$	$1\cdot 5—2 \times \cdot 5—\cdot 75\mu$
Flagella	5—10 peritrichous	1 polar	1 polar
Capsule	Conspicuous	None	Not recorded
Colonies on nutrient agar	Sub-circular, yellow	Greyish white to pearl grey	Circular yellow
Gelatine stabs	Liquefaction napiform	Liquefaction stratiform	Liquefaction filiform
Milk	Becomes slightly acid, casein precipitated with gradual extrusion of whey	Becomes intensely alkaline and clear without separation of casein	Becomes alkaline, casein is precipitated, whey clear
Test for indol	Positive	Positive	Negative

Résumé of Salient Characters.

Bacillus citrimaculans, n.sp. Causes spot disease of citrus, attacking fruit and branch, rarely found on leaves; attacks the parenchyma, first occupying the intercellular spaces then breaking down the cell walls; in nature attacks lemons, oranges and naartjes, and in addition has been successfully inoculated into limes, shaddock, grape fruit, citron; seville oranges are resistant.

A slender rod with rounded ends, 1—4 × ·45—·7μ, average 1·5 × ·5 to ·6μ; motile by 5—10 peritrichous flagella; conspicuous capsule but no spores; involution forms in broth containing high percentages NaCl; stains readily by usual stains and exceptionally well with gentian violet and by Gram's method.

Forms showing sub-circular yellow colonies on nutrient agar with dense, grumose centre; liquefies gelatine, clouds nutrient broth, forming pellicle and sediment; does not liquefy blood serum; coagulates milk with precipitation of casein and extrusion of whey, the coagulum is not redissolved; no growth in Cohn's solution; characteristic growth in Uschinsky's solution.

No gas in fermentation tubes, acid with dextrose, laevulose, galactose, maltose, saccharose and mannite, but not with lactose, glycerine, dextrin, or starch. Nitrates reduced with evolution of gas; tolerates up to 10 per cent. NaCl; no growth in broth over chloroform, indol produced in media containing peptone.

Aerobic, facultative anaerobe; killed by 60 minutes exposure to sunlight; optimum reaction about 20 Fuller; T.D.P. 62° C., maximum for growth 43° C.

Group number 221.2321523.

Summary.

This paper gives an account of a citrus disease which is causing considerable trouble in the Western Province of the Cape. It causes dark sunken spots on fruit and shoot, and not only disfigures but provides an entrance for fungous parasites which completely destroy the fruit.

The causal organism is described at some length, and as it appears to be one hitherto undescribed has been named *Bacillus citrimaculans*.

The question of preventive measures is not discussed, but an

improvement in the sanitation of the orchard would certainly prove beneficial, and it is intended to carry out spraying experiments during the ensuing season.

Botanical Laboratories of the Union of
South Africa, Pretoria.

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EXPLANATION OF PLATES.

All drawings were made with the aid of the camera lucida.

PLATE

III. Lemons, natural infection from Simondium, C.P., (*a*) lemon with a single typical spot, (*b*) the fruit on the right has been wounded by numerous scratches.

IV. Lemons, natural infection, from Simondium, (*a*) an early stage of infection, the spots are still light coloured. (*b*) Numerous infections at and remote from stalk coalesced to form irregular bodies.

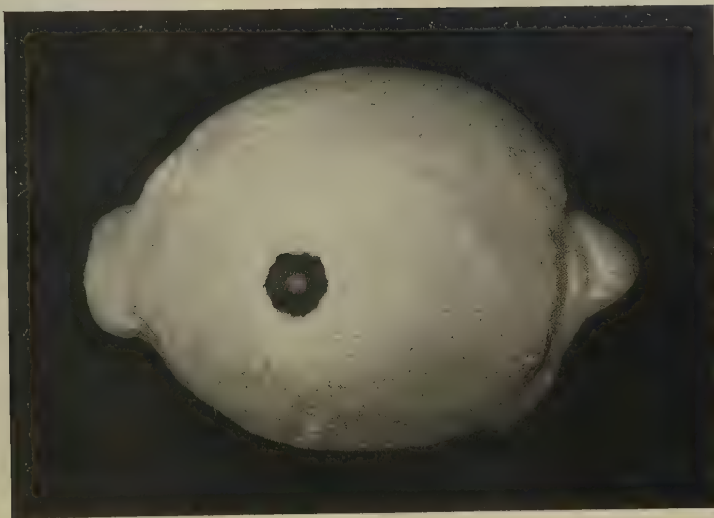
V. Lemons, artificial infection, pricked with sterile needle, then three of them atomised with suspension of a pure culture of *B. citrimaculans*; the fruit to the right in (*a*) is a control pricked with a sterile needle only.

VI. Lemons, artificial infection, ten days after inoculation, (*a*) inoculated by needle pricks, (*b*) with hypodermic syringe.

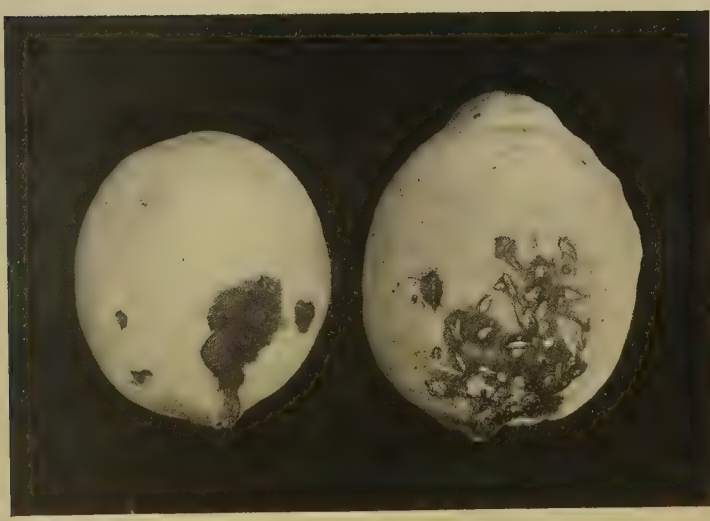
VII. Naartjes (*a*) artificially infected by needle pricks, (*b*) natural infection from Simondium.

VIII. (*a*) Navel oranges, natural infection from Simondium.
(*b*) "Parson Brown" seedling orange, artificial infection.

IX. (*a*) Lemon twigs showing infections round leaf bases, and petioles of fallen leaves.
(*b*) Small branch from orange showing the same characteristic discolorations.
(*c*) Leaves with bacterial spot, these are apparently rare and associated with tearing of tissues by thorns.



(*a*)



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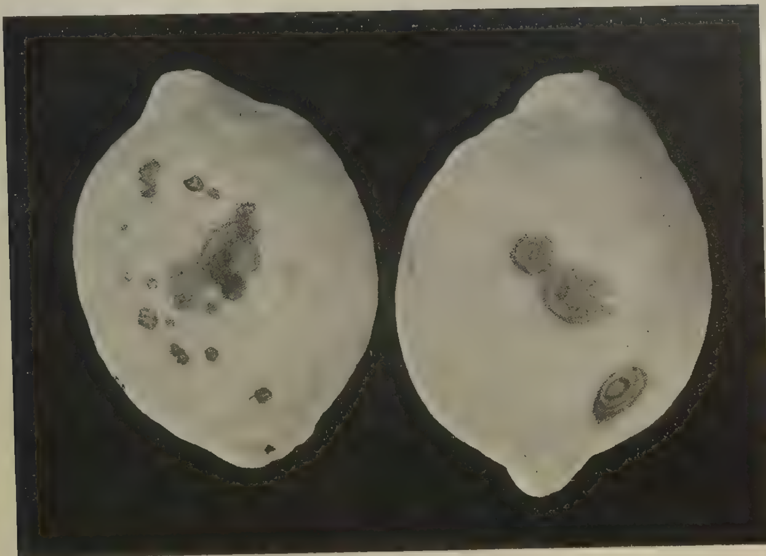

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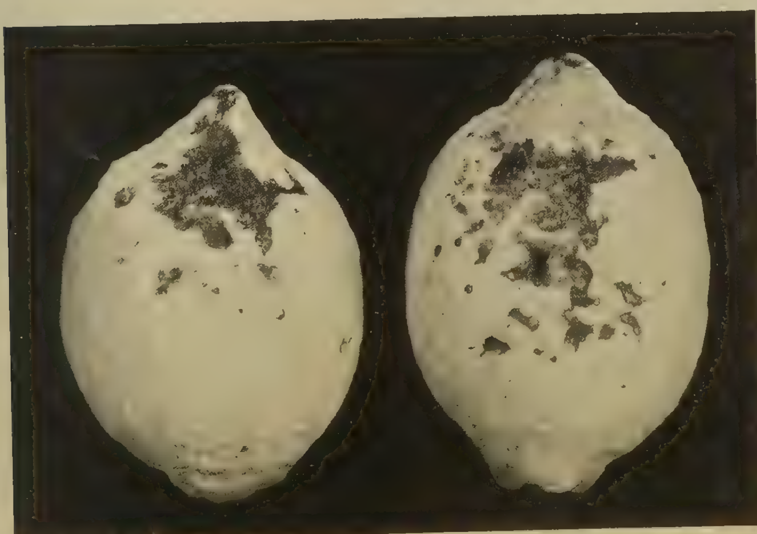

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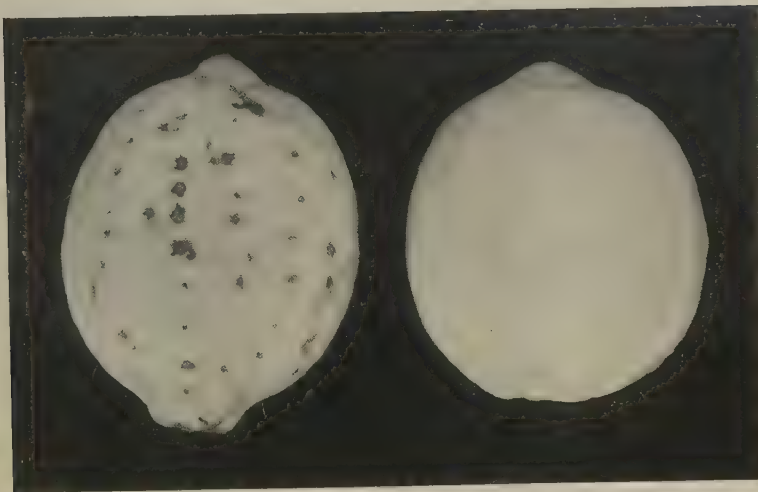

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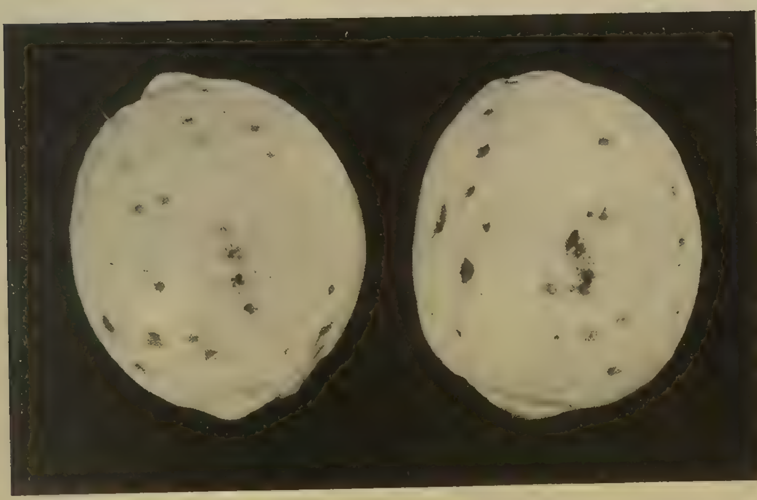

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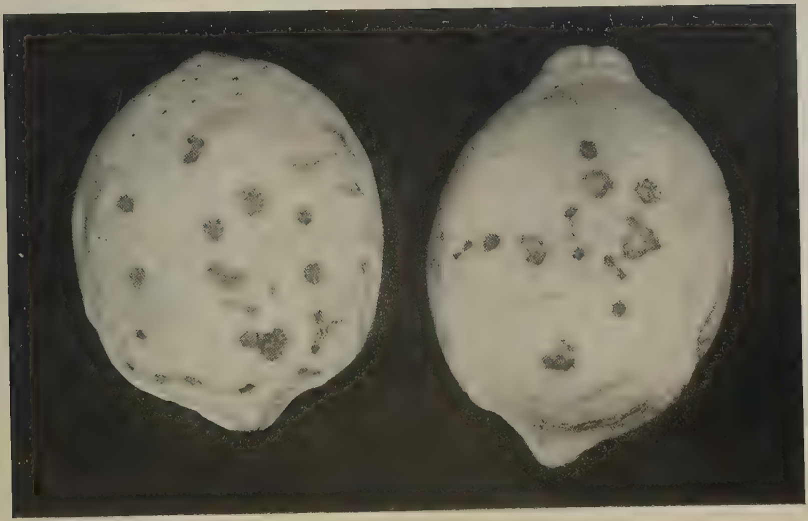

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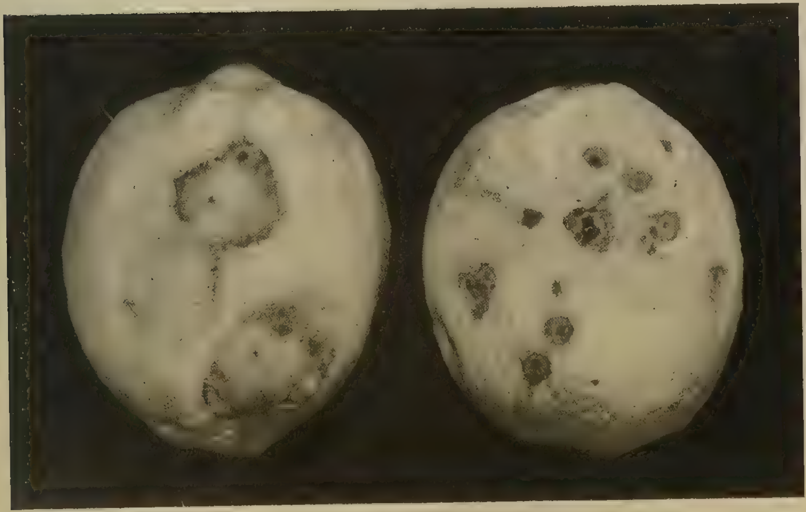

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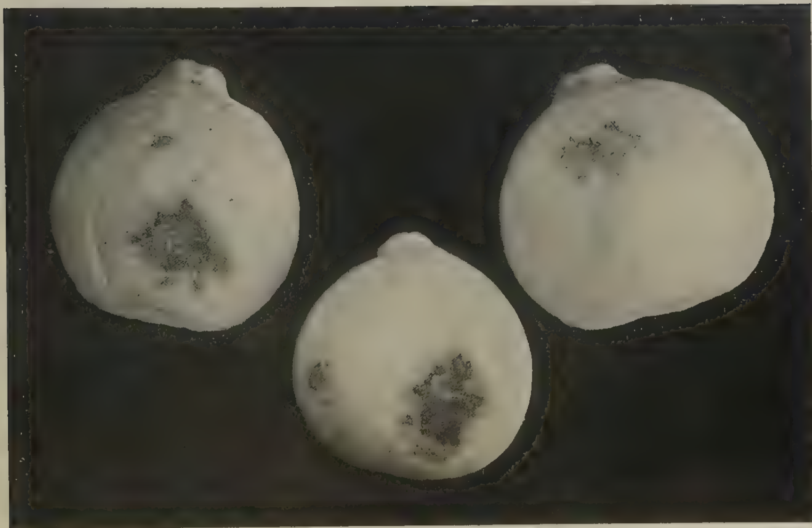

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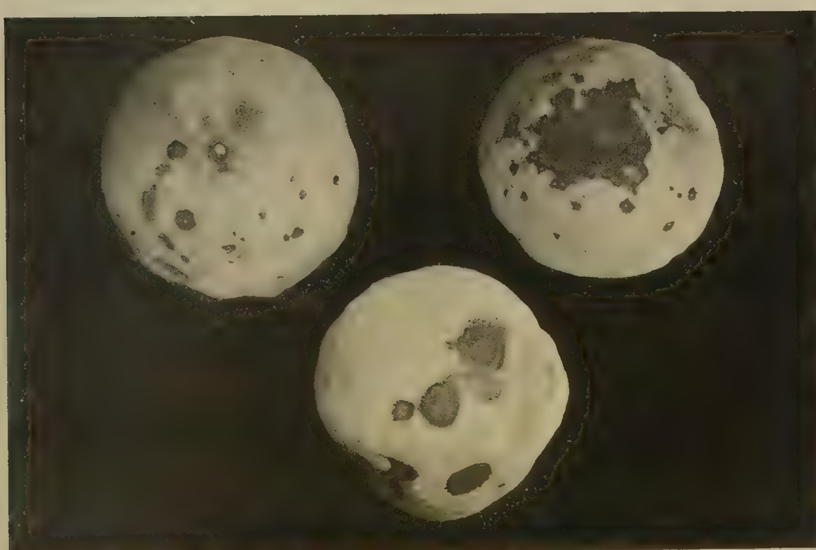

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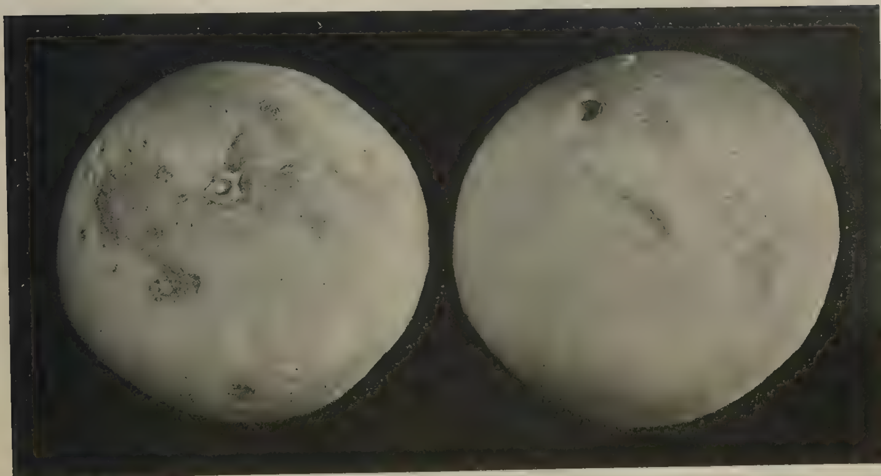

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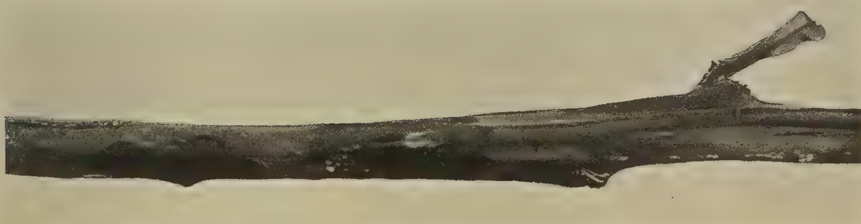

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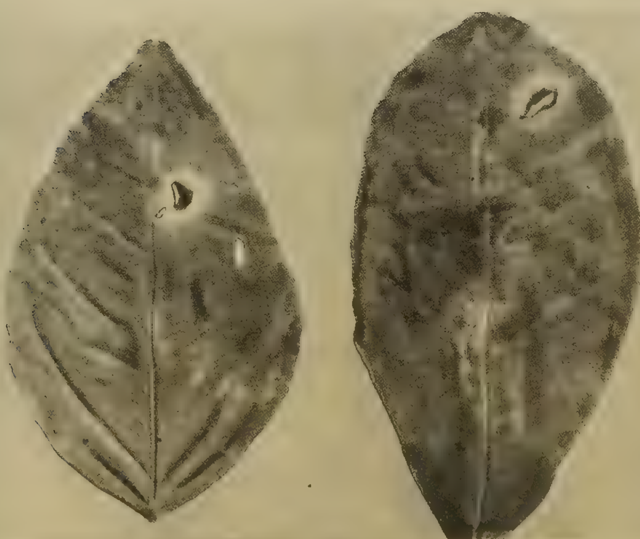

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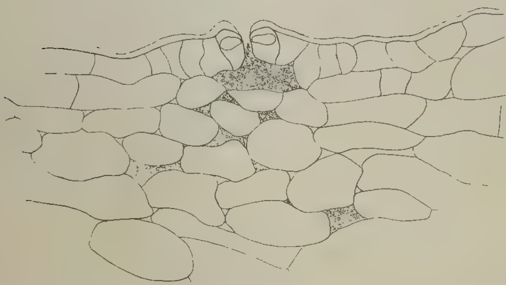

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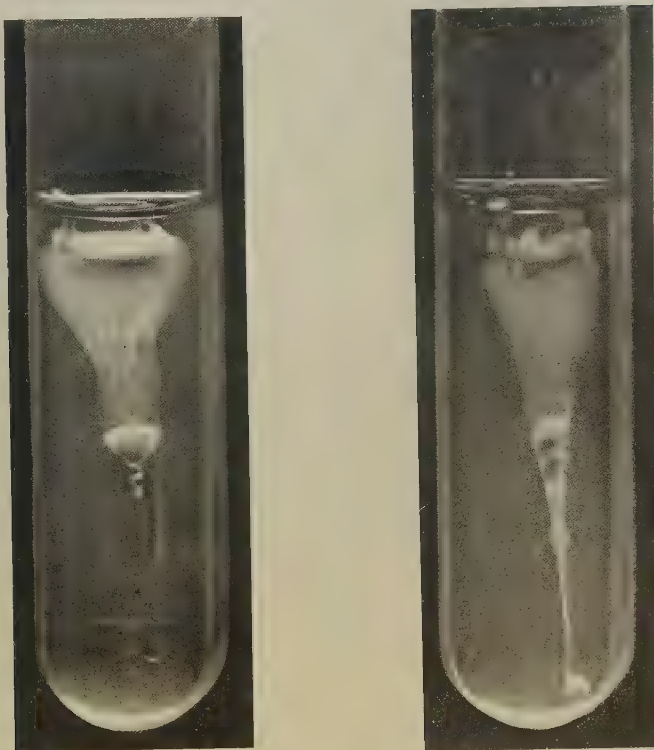

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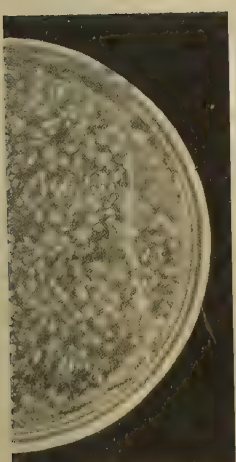

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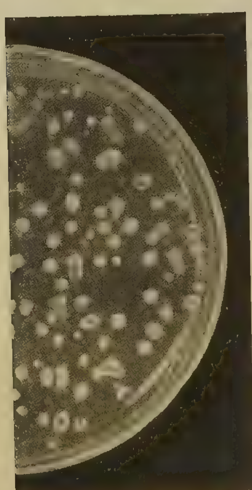

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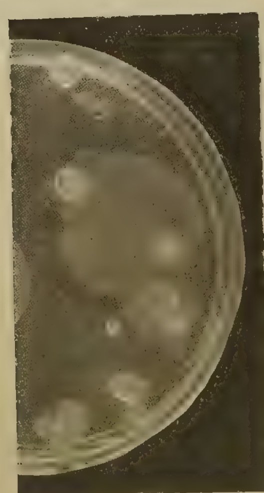

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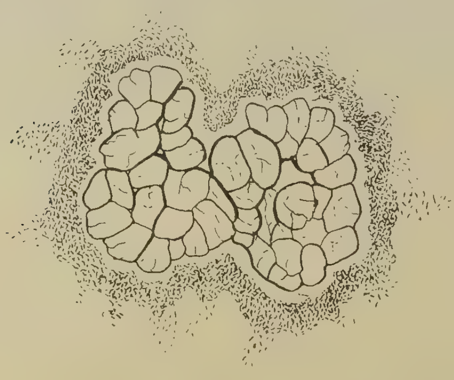

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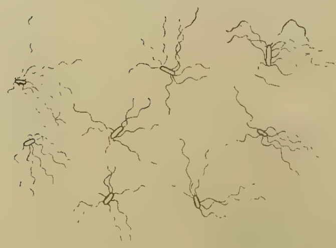

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X. (a) Section through naartje rind at an early stage of infection showing the bacillus in sub-stomatal cavity and adjoining intercellular spaces. The size of the bacilli is not indicated. Zeiss $\frac{1}{12}$ mm., comp. ocular No. 6*.

(b) Section through lemon rind, bacillus has multiplied and distended intercellular spaces: same magnification as (a)*.

(c) Section through tissues of infected orange rind, showing cells completely disintegrated and occupied by masses of bacteria. Magnification as above*.

XI. (a) Stab cultures in nutrient gelatine after 11 days at 20° C., and 2 days at room temperature (about 25° C.). (b), (c) and (d) colonies on nutrient agar at 25° C. (b) and (c) 3 days old, (d) 5 days old. Shows the effect of crowding on the size of the colonies—somewhat reduced, diameter of petri dish, $3\frac{1}{2}$ inches.

XII. (a) Development of organism on agar hanging block: for full explanation see text (Zeiss $\frac{1}{12}$ oil immersion, comp. oc. No. 12. This magnification used for all drawings in Plates XII and XIII unless otherwise stated)*.

(b) 24 hour colony on hanging block (comp. oc. No. 6).

(c) Rods showing flagella stained by Ellis' modification of Loeffler's method, 18 hours at 25° C.

XIII. (a) Chains from pellicle on laevulose broth, 16 days at 25° C.

(b) Aberrant forms on potato cylinders, 12 days at 35° C.

(c) Capsuled rods from edge of impression preparation of colonies on nutrient agar, 18 hours at 25° C., stained with fuchsin.

(d) Rods in slime oozing from fruit, stained by MacConkey's method.

(e) Involution forms from broth containing 7·5 per cent. NaCl, 48 hours at 25° C., stained with aniline gentian violet.

* Exigencies of space have necessitated the reduction of the following figures:

Pl. X (a) by $\frac{1}{2}$: (b) by $\frac{1}{2}$: (c) by $\frac{1}{3}$.
Pl. XII (c) by $\frac{1}{2}$.
Pl. XIII (a) by $\frac{1}{2}$: (c) by $\frac{1}{4}$. [ED.]

REPORT ON A TRIAL OF TARRED FELT "DISCS" FOR PROTECTING CABBAGES AND CAULIFLOWERS FROM ATTACKS OF THE CABBAGE-ROOT FLY.

By J. T. WADSWORTH,

Research Assistant in the Department of Agricultural Entomology, Manchester University.

(With Plate XIV.)

CONTENTS

INTRODUCTION.

THE present investigation has been carried out at the suggestion of Dr A. D. Imms. He has displayed a keen interest in the progress of the experiments and I have had the benefit of discussing with him any points that arose from time to time.

The expenses entailed during these experiments have been met from the annual grant of the Dept. of Agricultural Entomology, Manchester University.

It is a fact only too well known to most growers of cabbages, cauliflowers and related vegetables that large proportions of their crops are frequently lost owing to attacks of the Cabbage-root Maggot, the larva of *Chortophila brassicae*. A very large number of remedies have been devised with the object of either destroying the maggots

on infected roots, or preventing the deposition of eggs near the host plants. With one or two exceptions, however, these remedies are of little practical value since they require, as a rule, frequent application to the plants in order to obtain any satisfactory measure of success. For this reason, and also on the score of expense, most growers are averse to their use. Gibson and Treherne (6), in their recently published bulletin on the Cabbage-root Maggot, give a list of forty-eight insecticidal mixtures or protective measures which they have experimented with for several years. As a result of these experiments they make the following statement: "It can be truly claimed that the only protection to be relied upon for cabbages and cauliflowers and one which is in every way practical, is in the use of tarred felt paper discs."

The idea of placing paper collars round the stems of cabbage plants, and thus preventing egg deposition in the soil near plants so protected, originated with Prof. W. W. Tracy, of Detroit, Mich. in 1887. The material (manilla paper) which he used, however, was unsuitable for the purpose and the results of his experiments were unsatisfactory. In 1889 Prof. E. S. Goff of Wisconsin (7) substituted tarred paper for the material used by Tracy, with complete success. Goff also devised an efficient tool for cutting the discs expeditiously; the latter were about three inches in diameter, hexagonal in shape, with a slit extending from one angle to the centre and with a star shaped cut also in the centre.

Since Goff proved his method of protecting cabbages and cauliflowers by means of tarred discs to be thoroughly practical it has been widely adopted in America by commercial growers and others. The method has also been tested at almost all the Agricultural Experiment Stations in the United States and Canada in those districts where serious infestations of Cabbage-Maggots occur: with one or two exceptions the resulting reports have been entirely favourable.

The following authors have published reports of successful trials of tarred felt discs: Slingerland (11), Smith (12), Britton and Walden (3), Caesar (4), Schoene (8 & 9), Britton and Lowry (2), Gibson and Treherne (6).

On the other hand Schöyen (10), Blair (1), and Washburn (15) obtained unfavourable results in the trials which they made of tarred discs.

In these cases the lack of success is attributed either to placing the discs on the plants too late in the season, or carelessly, or to using tarred paper—which curls—instead of tarred felt.

The greater weight of opinion and evidence is distinctly in favour of the tarred disc method of protection and the majority of the above quoted observers agree that it is practical, economical and effective.

Apparently, however, there are no published records of any trials of this method having ever been made in this country. References in British horticultural literature, and in reports on insect pests, to the use of tarred felt discs are very meagre and not very encouraging.

As numerous complaints of damage and losses sustained by growers owing to the depredations of the Cabbage-Maggot are continually being received, together with requests for information how to deal with the pest in question, the desirability of testing here the method which has proved so successful in America thus becomes apparent.

During successive seasons in my own garden I have lost considerable numbers of cabbages and cauliflowers owing to attacks of the Cabbage-Maggot and I had already decided to test the American discs when Dr A. D. Imms suggested that I should test them on a fairly large scale in a local market-garden. Arrangements were therefore made with a market-gardener to rent from him a piece of land and the work was commenced in the spring of 1916.

Description of the Experiments.

The tarred felt discs were obtained from the United States where they are regular articles of commerce. They were not the hexagonal form usually figured and described but they were square, being $2\frac{1}{2}$ inches each way with only two slits—a long slit extending from the middle of one edge to a point half-an-inch beyond the centre of the disc, and a short slit three-quarters of an inch long crossing the long slit at right angles in the centre of the disc (Fig. 3).

Two separate pieces of land situated at Northenden, Cheshire, were rented from Mr Chas. Heywood, a market-gardener, who undertook to prepare the land and perform the necessary operations of cultivation, planting, etc. The tests were made on both cabbages and cauliflowers and the two plots of land were about sixty yards apart. The land on which the cabbages were planted was under cauliflowers in 1915 and the crop then suffered severely from maggot attack, from two to three-fifths of the plants being lost owing to this cause. This piece of land was selected in order to ensure, so far as possible, a heavy infestation of maggots.

A heavy dressing of well-rotted farm-yard manure was spread over the land towards the end of April; this was then ploughed under, the land afterwards harrowed down and then rolled in order to consolidate the soil—which is very light in texture—and to render conditions favourable for placing the discs as flat as possible.

(a) *With Cabbages.*

The cabbages (variety Leeds Market) were planted out on May 1st and the discs were placed in position on the following day. Altogether 816 cabbages were utilised for this experiment: they were planted in eight rows, each row containing 102 plants: disced and undisced rows alternating with each other. The spring was a particularly good growing season and the plants made excellent growth. On June 18th, some of the unprotected plants first showed signs of maggot attack and on June 23rd a count of the plants was made. None of the protected plants showed signs of attack but thirty-one of the unprotected plants—equal to 7·6 per cent.—exhibited the usual signs of severe infestation. The attack was very evenly spread over each of the four undisced rows: the numbers of attacked plants in each row being nine, seven, seven, and eight respectively.

On July 8th the cabbages were again counted when the percentage of attack had increased to 13·2; fifty-four out of the total number of 408 unprotected plants being severely attacked, whilst only one of the protected plants had succumbed to maggot attack, i.e. less than 0·25 per cent.

Several of the infected plants were taken up for examination and in all cases the original fibrous roots were absent, having been destroyed by maggots; in some cases the latter were still feeding on the roots and in those cases where they were absent pupae were present in the surrounding soil. In many plants new fibrous roots were developing near the upper portion of the main root and it was evident that the attack caused by the first generation of flies was ended.

The opinion of the market-gardener was that possibly one-third of the infected plants would develop a fresh set of roots and recover from the attack, although the resulting cabbages would be only small.

On July 13th the plants were counted again. No further attack was evident; the cabbages were counted at intervals of seven days until mid-August but no further losses were incurred.

As only one plant was lost owing to maggot attack out of 408 plants protected by the discs it is very strikingly evident that the latter afforded a very complete protection to the plants. The protected cabbages were also larger on the average and slightly earlier in coming to maturity than those in the unprotected rows. A probable explanation of these differences will be mentioned when discussing the results of the cauliflower trials.

Some alterations in the discs themselves were made and the method of placing them on the plants was also varied, as follows. On one row all the discs used were perforated with a small hole in the centre where the two slits cross, in order to obtain a closer fit round the stems of the plants. On another row two discs were applied to each plant, the discs being crossed so that when the slits are forced open by the stem thickening during growth, the soil is not exposed, as it is to some extent when single discs alone are used. Where two discs were used it was surmised that female flies would be absolutely prevented from gaining access to the soil near the plants so protected. As will be seen from the results obtained the single discs gave quite adequate protection and no corresponding advantage was gained by using either perforated discs or double ones to compensate for the additional expense and trouble entailed.

(b) *With Cauliflowers.*

The land on which the cauliflowers were planted was similar in character to that on which the cabbages were grown; it was prepared in the same manner and was cropped with sage the previous season. The seedlings (variety Autumn Giant) were planted out on May 27th and the discs were placed round the plants immediately after planting. The latter were planted in four rows, each row containing 233 plants, i.e. 932 plants altogether. The protected rows alternated with the unprotected ones: rows I and III being protected, rows II and IV were unprotected.

The first count was made on June 23rd with the following result. On row I adjoining the field (see Fig. 1) no plants showed signs of maggot infestation; row II, sixty-two plants attacked; row III one plant infected; row IV, ten plants had succumbed.

Altogether seventy-two of the 466 unprotected plants were attacked, equal to 15·4 per cent., whilst only one of the protected plants was attacked up to that date.

On July 4th the plants were again counted when considerably further losses had occurred; a photograph (Fig. 1) was taken on this date.

The results of the countings are given in tabular form; a table showing the results obtained with the cabbages is also given for comparison.

No further cabbage plants were destroyed by maggots on this plot after July 8th. 768 cabbages, out of a total of 816 planted, had been

cut and marketed up to the end of September. This number agrees closely with the number of plants (761) unaffected with maggots on July 8th. A few plants had evidently recovered and made marketable plants by the end of September, as predicted by the market-gardener.

Table showing the numbers of Cauliflower plants destroyed by Cabbage-root Maggots when protected by tarred felt discs, and when unprotected. Each of the four rows contained 233 *plants.*

Dates when counted	Row I protected	Row II unprotected	Row III protected	Row IV unprotected	Results in percentages: protected rows (I and III)	Results in percentages: unprotected rows (II and IV)
June 23	0	62	1	10	—	15·4
July 4	0	132	7	56	1·5	40·3
July 15	5	141	10	85	3·2	48·5
Aug. 5	11	174	13	120	5·1	63·0

Results with Cabbages. Each row contained 102 *plants.*

Dates when counted	Rows II and IV protected	Rows I and III unprotected	Rows VI and VIII protected	Rows V and VII unprotected	Results in percentages: protected rows II, IV, VI, VIII	Results in percentages: unprotected rows I, III, V, VII
June 23	0	16	0	15	0	7·6
July 8	1	33	0	21	0·2	13·2

The cauliflower plants were also counted on July 8th but no further losses had occurred since the previous count (July 4th). The effect of the infestation produced by the first brood of flies had reached its maximum about this date.

From July 15th onward the losses increased very considerably as will be seen by reference to the table. This increase was attributed to a secondary attack of maggots produced by the second generation of flies. At this period also a spell of three weeks of very hot, rainless weather prevailed; these weather conditions rendered the cauliflowers more susceptible to maggot attack and less able to recover therefrom.

On August 12th the plants were again counted but no further losses were observed; on August 16th some of the cauliflowers were ready for cutting and counting was discontinued.

The results obtained prove very conclusively that the tarred felt discs are a very effective means of protection from cabbage maggot attacks. Only twenty-four cauliflower plants were lost out of a total number of 466 protected by discs, whereas 294 plants were lost out of

the same number of unprotected ones. A net gain of 57·9 per cent. was thus effected by using the protective discs.

The results obtained with the cabbages were even more striking as only one plant was lost out of 408 protected by the discs. The infestation, however, was not nearly so severe in the cabbages as in the cauliflowers. Only 13·2 per cent. of the unprotected cabbages were lost as against 63·0 per cent. of the cauliflowers.

Market-gardeners, and gardeners generally, hold the opinion that cauliflowers are much more liable to be destroyed by maggot attack than cabbages, and the results obtained in these trials give strong support to this view. Whether it is a fact that cabbage-root flies are more strongly attracted to cauliflowers than they are to cabbages, or whether the former plants succumb more readily to maggot attack than do cabbages, remains a subject for further enquiry. The above results clearly show that, on similar land, cauliflowers suffered much more severely from root-maggot infestation than cabbages. The only factor that differed in the two cases, was that the cabbages were planted out a month earlier than the cauliflowers, and consequently were well established, and growing vigorously, before the maggot attack became severe.

Gibson and Treherne, who discuss very fully this question of the relative susceptibility of cauliflowers and cabbages to maggot attack, make the following statement:

"We consider that the supposed susceptibility of cauliflowers over cabbages is probably due to the lesser vitality of the former plants."

During a period of seventeen days between June 25th and July 11th, 1915, at Agassiz, B.C., 1418 eggs were deposited on six cabbage plants, while only 1038 were laid on six cauliflower plants; these results lead them to believe that no special choice exists on the part of the fly to deposit on cauliflowers over cabbages. On the other hand, in the table given on p. 26 of their bulletin, they state that 6602 eggs were laid on twelve cabbage plants during 133 days, an average of 550·1 per plant, equal to four eggs per plant per day. On six cauliflowers 5515 eggs were laid during 117 days, an average of 919·1 eggs per plant, equal to seven eggs per plant per day; i.e. nearly twice as many eggs were laid on cauliflowers as on cabbages. Although the periods of time were not coincident—the countings of the eggs on the cauliflowers being made later in the year than in the case of the cabbages—the results would appear to indicate that the flies were more strongly attracted to the cauliflowers than to the cabbages.

Precautions to be observed in applying the discs, and other remarks.

In order to obtain the best results from the use of tarred felt discs it is advisable that the soil should be in a fine friable condition when the discs are placed in position, and further, that the plants should not be placed in depressions of the soil but if possible on a slight ridge. If the soil is lumpy the discs will not lie flat on the ground and the female flies will then be able to crawl underneath the discs to deposit eggs. If the discs are placed below the soil level they are liable to become covered with soil after rains, which renders them less efficient. It is also of the utmost importance to place the discs round the plants immediately after planting out, if this operation is performed later than the first week of May. In the district around Manchester, eggs of the cabbage-fly were found on May 14th in 1915, and on May 18th in 1916; these were the earliest dates they were found in those seasons. Probably in the South of England they would be found much earlier, and in the North somewhat later. On seedlings planted out earlier in the year, it is not so important to place the discs in position immediately after planting, although there may be advantages to be gained by doing so.

Several of the observers, who have tested the tarred felt discs, draw attention to the fact that protected plants are usually larger, and mature earlier, than unprotected ones. These differences are rightly attributed to freedom from root injury owing to the absence of maggots on the roots. Another possible factor, which contributes to this result of increased size and early maturity, may be referred to here. If the soil underneath the discs is examined on hot days, during the first two or three weeks which follow the planting out of the seedlings, it will be found in a moist condition, whereas the soil round the unprotected plants will be dry. The disc, by preventing evaporation from the surface of the soil, conserves the moisture in the soil directly beneath it. The plants are thus enabled to recover more quickly from the effects of transplanting, and the conserved moisture also helps them to withstand better the effects of prolonged drought. More especially is this the case with regard to cauliflowers, which suffer more from drought than cabbages.

The question is frequently asked whether the discs, owing to the smell of the creasote with which they are impregnated, deter flies from

laying eggs. Whilst the creasote may exercise a deterrent influence on the flies for a short period of time after the discs have been placed round the plants, this influence—supposing it to exist—soon disappears. Frequently eggs may be found both on the surface of the discs, and also underneath them: a fact which tends to disprove the idea that flies are deterred from laying eggs owing to the emanation of odours from the discs themselves. The chief function of the disc is to act as a mechanical obstacle to the efforts of the flies to deposit eggs in the soil near the plant.

Growers have also asked for information regarding the action of the discs on slugs. No definite experiments have been made with the object of determining the effects of the discs with regard to slugs. It is worthy of note however that, in the row of 233 cauliflowers adjoining the field of clover (see Fig. 1), only two plants were definitely proved to have been injured by slugs. As numerous slugs were observed in wet weather along the edge of this field, it would appear probable that they do avoid the discs. Occasionally a slug was found sheltering beneath a disc, but this was where the ground was lumpy and spaces were present underneath the disc. If the discs are pressed closely to the soil, no accommodation would remain for slugs, and they would thus be prevented from crawling underneath.

Several market-gardens were visited during the summer months in order to determine the relative proportion of plants destroyed by maggot attacks on different types of soil. An interesting fact was observed in a market-garden situated about two miles from the one where the above described experiments were conducted. Although I examined hundreds of cauliflowers there, it was difficult to find a single plant infected with root-maggots. The soil is a heavy clay loam—the owner described it as a good strong soil—and the gardener attributed to this fact his immunity from root-maggot attacks. He also uses gas lime abundantly, having a strong belief in the beneficial effects of this substance against root-maggots and pests generally. The contrast between the two cauliflower plots, at the end of July and early August, was very striking. On the one plot, where the soil was light in character, from fifty to sixty per cent. of the cauliflowers were destroyed by maggots: on the heavy soil, hardly a single plant, in three or four hundred, was lost from this cause. All the growers, with whom I discussed the matter, agreed that cauliflowers and cabbages are more liable to become infested with root-maggots when grown on light soils, than they are when grown on heavy soils. Various reasons were given in support of this opinion, and

without attempting here to discuss this aspect of the question, I may say that all the evidence obtained during the summer in this connection supports their view.

Trials of the discs have also been made with winter-greens and flowering broccoli. Very little advantage has been apparent with regard to these plants up to the present time (end of September). Probable reasons for this fact being that the intensity of attack of the second brood of flies was declining about the time when planting out took place, and also because at this period (end of July and early August) the greatest numbers of *brassica* plants are growing in the fields; consequently the numbers of eggs laid near any single plant are not likely to be so numerous as when fewer plants are in the fields and gardens, as is the case earlier in the season.

As stated above, tarred felt discs are regular articles of commerce in America, where they are largely used by growers. The discs used in these experiments were obtained from A. B. Cowles, 25, S. Water St., Rochester, N.Y.; they cost two dollars per thousand (120 for a shilling) in addition to carriage. In this country at the present time tarred felt discs are not obtainable commercially. Various kinds of materials, however, have been tested this summer and arrangements are in progress whereby, it is hoped, suitable discs will be purchasable at a reasonable price in England. Growers who suffer losses from root-maggot attacks will thus be enabled to test for themselves the value of this method of protection.

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DESCRIPTION OF PLATE XIV

Fig. 1. Photograph showing appearance of the cauliflower plot on July 4th. Rows I and III (counting from the field on the left side of the photograph) were protected with tarred discs, rows II and IV were unprotected. The remaining rows of plants were not utilised in these experiments.

Fig. 2. Photograph of a brussels-sprout plant showing a disc in position.

Fig. 3. Outline figure of a disc; actual size.

Fig. 1

Fig. 2

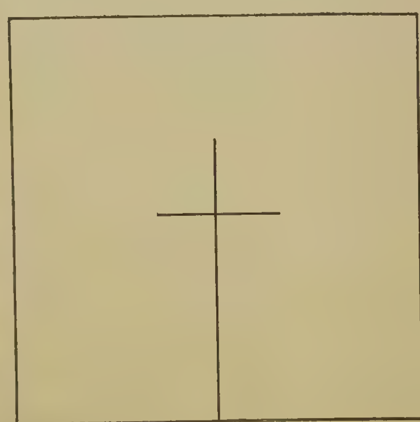

Fig. 3

ON THE RESISTANCE TO FUNGICIDES SHOWN BY THE HOP-MILDEW (*SPHAEROTHECA HUMULI* (D.C.) BURR.) IN DIFFERENT STAGES OF DEVELOPMENT.

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(With Plate XV.)

In the testing of the resistance of any fungus to a chemical substance both the environmental conditions and the stage of growth of the fungus are, obviously, important factors.

We know, *e.g.* from Clark's[1] experiments, that the spores of a fungus when supplied with nourishment in the form of a decoction of sugar-beet show a greatly increased power of resistance to the toxic effects of copper sulphate. With *Œdocephalum albidum* the lethal strength for spores in water is ·0013 %, whereas with spores supplied with nourishment it is 0·1 %,—about 80 times as strong.

In a recently published paper[2] dealing with the fungicidal action of certain chemicals, principally sulphides, attention was drawn to the fact that *data* as to the behaviour of spores placed in a fungicide have little practical value as indicating the strength at which the same chemical will be fungicidal when used against the growing fungus on the plant. It was pointed out that while Foreman[3] found that a 0·16 % solution of caustic soda prevents the germination of spores of *Botrytis cinerea* and *Sphaerotheca mors-uvae*, experiments have shown that a 0·3 % solution does not kill well-developed patches of the conidial stage of *S. Humuli*.

In all our spraying experiments with "powdery mildews" (*Erysiphaceae*) carried out during 1914 and 1915 we were careful to select for spraying well-developed patches of mildew showing dense clusters of conidiophores bearing chains of conidia—a stage of development

[1] *Bot. Gazette*, Vol. XXXIII (1902).

[2] J. Vargas Eyre and E. S. Salmon, "The Fungicidal Properties of Certain Spray-fluids, *Journ. Agric. Science*, Vol. VII, 473 (1916).

[3] Foreman, F. W., "The Fungicidal Properties of Liver of Sulphur," *Journ. Agric Science*, Vol. III, 401 (1910).

which may be briefly described as a "powdery" patch. This stage was chosen both in order to make the experiments as strictly comparable as possible and on the assumption that in such a stage of development — where the fungus has a well-developed mycelium furnished with very numerous haustoria—the resistance to the fungicidal properties of the chemical would be at its highest.

During experiments this season (1916), however, facts have been observed which show that the mildew when in an earlier stage of development on the host-plant is more difficult to kill. Our suspicions of this fact were first aroused by the occurrence of minute tufts of conidiophores at fresh places on the leaf two or three days after the spraying had taken place. These fresh little patches of mildew could not have been due to re-infections subsequent to the spraying, since a conidium takes about five days from infection to develop mycelium and conidiophores. The point was then studied more closely by means of hop-leaves inoculated with conidia of the hop-mildew. It was found that very soon after being inoculated the young hop-leaf, when rapidly growing, shows small raised "blisters" or "humps" at the places where it has been infected. At first the raised blister is green all over, and shows no trace to the naked eye of a white mildew, although usually, under a lens, a few delicate hyphae may be seen radiating from the centre of the "hump." After a day or two, each "hump" shows a whitish growth beginning to spread over its surface, due to the formation of branched mycelial hyphae, and soon afterwards the first conidiophores appear. An exactly similar appearance may be found on a young Rose-leaf when attacked by the Rose-mildew (*S. pannosa*).

Spraying experiments on hop-leaves bearing numerous "humps" showed that the mildew in this very early stage of development has a greater power of resistance to sulphide solutions. Thus in experiments it was found that a certain stock solution of ammonium sulphide when diluted 1 to 100 with water was completely fungicidal to the hop-mildew when in its "powdery" conidial stage; this solution did not, however, prevent the youngest stage of the mildew, described above, from developing and forming conidiophores. At double the concentration however, the sulphide solution in question became fungicidal for this earliest stage of development.

The details of two experiments may be given here. In *Exper.* 1 the fungicide used was a certain concentrated solution of ammonium sulphide diluted 1 to 100 with water (and containing 1 per cent. soft soap). Three seedling hop-plants were used, and the leaves on each which were sprayed bore the hop-mildew in the stages of development noted below:

plant *a*: leaf at 5th node (from apex) bearing numerous "powdery" patches.
,, 4th ,, bearing "humps," showing a few very young sterile hyphae.
plant *b*: ,, 5th ,, bearing numerous "powdery" patches.
,, 4th ,, bearing "humps," showing a few young sterile hyphae.
plant *c*: ,, 5th ,, bearing numerous "powdery" patches.
,, 4th ,, bearing green "humps" which showed under a lens no signs of any hyphae.

All the plants bore at the respective nodes two leaves, each with the mildew in the same stage of development, and one leaf at each node was left unsprayed as a control. The spraying (using an atomiser) was carried out on June 6. By June 10 it was apparent that on all the sprayed leaves where the mildew had been in the "powdery" conidial stage the ammonium sulphide solution had almost or quite killed the fungus; in every case, however, the leaves which bore the humps showed the mildew in a further stage of development. On plant *a* there were numerous tiny tufts of conidiophores appearing from the "humps"; on plant *b* there were two such tufts of conidiophores, and on plant *c* nine such tufts. By June 17 the original "powdery" patches on the three sprayed leaves were all dead and in many cases semi-obliterated; the control leaves all bore very numerous almost continuous densely powdery patches. The sprayed leaves with the "humps" were as follows: *a*, ten very small though densely powdery tufts had developed over the "humps"; *b*, seven small densely powdery tufts of conidiophores had developed where the youngest "humps" had been,—where the "humps" had shown, at the time of spraying, mycelial hyphae, the fungus had been killed; *c*, twelve tiny powdery tufts of conidiophores had developed over the "humps." In every case the control leaf showed much more numerous and much larger densely powdery patches of conidiophores. It was clear from this experiment that the ammonium sulphide solution used was fungicidal for the mildew in the "powdery" conidial stage, but was not fungicidal for the mildew in its earliest stages of development, although it appreciably checked its growth.

In *Exper.* 2 the fungicide used was the same concentrated solution of ammonium sulphide diluted 1 to 50 with water (and containing 1 per cent. soft soap). The seedling hop-plant used bore at the 5th node (from the apex) two leaves, each bearing very numerous "powdery" patches of mildew, and at the 4th node two leaves, each bearing from thirty to forty "humps," only a few of which showed young mycelial hyphae on their surface. One leaf at each node was sprayed on June 6; the remaining leaves served as controls. On June 10 the "powdery" patches on the sprayed leaf were all dead and semi-obliterated; the "humps" on the sprayed leaf showed no signs of any living mildew. On June 12 the control leaf at the 5th node bore numerous densely powdery patches, while the sprayed leaf showed no signs of any living mildew; at the 4th node the sprayed leaf bore about thirty "humps" with no trace of mildew developing from them, while the control leaf bore over thirty "humps" each of which now bore a small densely powdery patch of conidiophores. On June 20 the sprayed leaf at the 4th node was of a healthy deep green colour and still showed the original "humps" but no trace of mildew occurred anywhere on the leaf; a photograph of this leaf as it appeared at this date is reproduced in Plate XV, fig. 1. The control leaf at the 4th node now bore densely "powdery" patches covering over the original "humps"; a photograph of this leaf is shown in Plate XV, fig. 2[1].

[1] I am indebted to Mr H. Wormald for taking these photographs.

Further, some evidence has been collected showing that the age of the mildew even when in the "powdery" conidial stage is a factor of importance. The older conidial patches have less power of resistance to the soluble sulphide spray than the young patches.

The explanation of the difference in resistance to fungicides shown by the earliest and the later stages of development cannot be given until further *data* are available. It would appear that to some extent the age and condition of the host-leaf are concerned; the mildew has less resistance in its conidial stage when on an old hop-leaf than when on a vigorous young leaf. But that this is not the whole explanation is shown by the fact, mentioned above, that on one and the same leaf a sulphide solution will kill the mildew where present in its well-developed conidial stage and fail to kill it in its youngest stages of development. It is possible that the earliest stage of development is more resistant because the first-formed haustorium from the appressorium of the germ-tube has more vigorous powers of resistance than the later-formed haustoria, or because the appressorium offers powers of resistance; on the other hand it may be that the vigorously growing conidial stage is less resistant through the formation of thinner-walled hyphae, or that the great number of haustoria present allows the fungicide to reach more easily the epidermal cells of the host-plant and cut off the food-supply.

On the old hypothesis that the sulphide solution acts fungicidally by virtue of the sulphur deposited, it could be held that the "powdery" conidial patches are killed because a sufficient amount of the sulphide solution is collected round them to give the requisite deposition of sulphur, while this would not take place in the case of the earliest stage of development when there are few mycelial hyphae and no conidiophores. We are unable to accept this explanation, however, since our work of last year has led us to the conclusion that with regard to this class of fungicides the sulphides contained in solution act as such and not by virtue of the sulphur deposited.

Whether it will be more economic to attempt to kill a "powdery mildew" by spraying at a strength lethal to its earliest stage of development, or to wait until the mildew has developed into the less resistant conidial stage, must be decided by future experiments in the laboratory and field. It is clear, however, that in the treatment of "powdery mildews" generally this difference of susceptibility at different stages of development must be kept in mind as a new factor of importance.

Fig. 1

Fig. 2

OBSERVATIONS ON THE LARVAL AND PUPAL STAGES OF *AGRIOTES OBSCURUS*, LINNAEUS.

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(*From the Department of Agricultural Entomology, Manchester University.*)

(With Plates XVI and XVII and 1 Text-figure.)

CONTENTS.

1. Historical.

Although the larval stage of *Agriotes obscurus* has undoubtedly been of economic importance for a long period, yet there does not appear to have been a good description of it until Westwood published a brief account in 1839. Marsham in 1808 (12) had figured a larva which he considered to be a stage of *Elater segetis* (= *Agriotes lineatus*), but the figure is too indefinite for accurate determination. Furthermore, he says he was unable to breed out the perfect insect, and was relying on Bierkander for his information.

Westwood (18), however, describes the larva of *Agriotes obscurus* and figures the antenna, the under side of the head and prothoracic segments,

and the first maxillae and labium; his figures are too small to be of much utility. He further notes that the larva had been figured previously by De Geer and Bierkander; but points out inaccuracies in the figures of these authors, that seem to indicate faulty identification.

Chapuis and Candèze in their work published in 1855(3) do not describe the larva, but merely quote references to the writings of Marsham and Westwood. In 1860, Curtis published in his work on *Farm Insects*, an account of Elateridae of importance to agriculture; he did not describe the larva of *Agriotes obscurus*, and considered the larva of *A. lineatus* to be the common and important species.

Schiodte(16) defined larval characters of the genus *Agriotes* in 1869, and described those of *A. lineatus*. He figured the left mandible, as well as the eighth and ninth abdominal segments. No mention is made of *A. obscurus*. Perris in 1878(14) described many larvae of Elateridae; but he merely quotes references to Marsham(12) and Westwood(18), and does not add any description of *A. obscurus*. Beling(2), in a full account of the metamorphoses of Elateridae, published in 1883—4, describes the larva of *Agriotes lineatus* very completely, and states that he could only detect slight differences between the larvae of *A. lineatus* and *A. obscurus*. Finally, the subject is brought up to date by Xambeu(20) in 1912 and 1913 by his work on the life histories of Elateridae. He adds little to our knowledge of *Agriotes obscurus*, merely referring to Beling (*op. cit.*) and adding a few descriptive remarks. It has been somewhat difficult to trace literature definitely referring to *Agriotes obscurus*, owing to the long established custom of terming Elaterid larvae under the collective title of "wireworms," without reference to any definite species. This, together with a number of cases of inaccurate identification in the older literature, has often rendered the information of doubtful specific value.

2. Material and Methods.

The idea of studying the life history of certain Elateridae, whose larvae are known as "wireworms," was first suggested to me by Dr Imms, to whom my thanks are also due for advice given from time to time. The first problem requiring solution, was the determination of the exact species of which the common wireworm forms the larval stage. With this intention, the material was purposely obtained from as wide a range of localities, and under as different conditions (of soil, crop, etc.), as possible. The area covered was mainly in Cheshire and around

Manchester, while a fair number of specimens were taken in various districts of Mid-Lancashire and North Staffordshire. Wireworms were taken in soils growing a variety of crops, but the most abundant and sure source of specimens was found to be the potato crop; a visit to a potato field rarely proving a disappointment. Pastures, especially those that had been down for a long period, proved a good source for material. They were, however, only utilised until sufficient observations had been made, owing to the lengthy examination required and the uncertainty of results. The material when collected was put into fairly deep tin boxes, loosely packed with damp soil. The larvae were examined individually under a binocular microscope in the laboratory, being measured and roughly classified into sizes. They were placed, after examination, in plant pots previously prepared. As this type of breeding cage proved to be useful, no trouble from disease having been experienced, some further details may be of interest. The drainage hole of the plant pot was covered with a circular piece of fine wire gauze strengthened by having a strip of zinc soldered round the edge. The gauze was roughly two-thirds the width of the pot; so, no matter how it slipped, the gauze would still cover the drainage aperture and prevent the escape of any larvae. The top of the flower pot was covered over by a perforated zinc plate, having a flange of about an inch in depth. The flange and the weight of the zinc were sufficient to keep the pot covered securely, even in the most boisterous weather. The vessel was filled first with small pieces of broken pots, and then with earth in the usual manner; a potato was placed about half way down in the soil to provide food for the larvae. The pots were buried out of doors in the ground, up to the level of the soil inside the pot. The larvae lived quite healthily in these pots, and no sign of disease was ever observed. For experiments requiring a greater depth of soil, two ordinary three-foot drainpipes were used; the narrow end being capped with cement, which was sloped inside to a central aperture covered by wire gauze imbedded in the cement. These drainpipes were very useful, but were too weighty to handle easily. They were handicapped by being buried in stiff clay soil, which prevented good drainage and rendered the drainpipes liable to become waterlogged. Buried in soil of a more open texture and better drainage capacity, they would be quite free from any disadvantage, save that of weight.

Throughout this work, the endeavour was to keep all the conditions as closely approximating to nature as possible.

The greater part of the laboratory work has been carried out in the Department of Agricultural Entomology of Manchester and I am also much indebted to Professor Hickson for affording me various facilities. Acknowledgment has already been made to Dr Imms. I am also indebted to Mr J. T. Wadsworth for the photograph of the pupal cell with the pupa *in situ*. Mr J. C. F. Fryer of the Board of Agriculture has kindly given me information as to distribution, etc., of the common wireworms. The work has been carried out during my tenure of a Board of Agriculture studentship and completed during the following year.

3. Notes on the Biology of the Larva.

The larval stages of Elateridae are practically ubiquitous, occurring in all types of soil. The larvae of *Agriotes obscurus* have been found most commonly in the lighter types of soil, particularly loams rich in organic matter. The range of soils that they inhabit is extraordinarily varied, including the grades between fairly stiff clay to light loam. They are practically always to be found in pastures, even when on clay soils, though in such a case they are much less plentiful. In Cheshire and Lancashire the common wireworm is the larva of *A. obscurus*, as was demonstrated by breeding experiments. This seems to be corroborated by the fact that Newstead records adult *A. obscurus* (when in a state permitting of identification) far oftener than that of *A. lineatus*, as being the common species taken as food by wild birds over the same area. Very probably, *A. obscurus* is the common species in the north of England; while *A. lineatus* seems to be the most common species further south, though *A. obscurus* and *A. sputator* are not far behind in point of numbers. An interesting feature with regard to *A. obscurus* is, that the occurrence of its larvae seems to be, in some way, closely connected with the presence of organic matter in the soil. The larvae are most abundant in soils containing a large percentage of humus, due either to the peaty nature of the soil, or to heavy manuring. The same thing was found in cottage gardens, which are usually very rich in organic matter. This point will be considered later in connection with the feeding habits.

The larvae of *A. obscurus* were found in what may be roughly described as three stages. These stages were, of course, not sharply defined, but merged into one another. They may be given approximately:

Small stage (1) 7 mm. long by 0·75 mm. broad.

Medium stage (2) 10—15 mm. long by 1·0—1·25 mm. broad.

Large stage (3) 17—23 mm. long by 1·5—2·0 mm. broad.

It should be stated that no living specimen was ever found to be more than 21 mm. long, and that 19 mm. is about the average length, taking a number of specimens into consideration. The 23 mm. specimen was an extended spirit example. After about a year, the small stage was no longer present, and only medium and large stages could be found in the plant pots. According to most authorities the larval period is supposed to last five years. From observations on the length and rate of growth, I am inclined to place the period at four years rather than five. The small stages, taken in one year from July to October, varied much in size. It was found, after a couple of months (November December), that a number of these apparently small specimens were really of medium size. It appears from this that the breadth is a safer criterion than the length (which is difficult to ascertain on the living larva). Judging from the size of specimens taken from July to October, and also from the fact that the adult beetles are common from April to June, it may be assumed that the newly hatched larva commences its free existence in July. Again, there are three larval moults, performed in the usual manner, by splitting the chitinous skin along the thorax, and leaving the old exo-skeleton behind. From these facts, and from the rate of growth, it may be assumed that the larva is full grown in three years. Full size larvae are found actively feeding up to October; but these same larvae apparently do not feed again (at any rate on vegetation), and pupate the next summer. It is thus probable that there are three stages limited by the three moults, and occupying three years. Then there is a period of active feeding, followed by a quiescent condition terminating in pupation, all this taking place in a year. It was found that of the large larvae (17 to 23 mm.) a number fed through the winter, while some were not feeding after October, and were not lethargic; these latter were broader than were the former. This would appear to support the above theory. If the life of the larva is one of five years, then it would seem that the early (small) larval stages would continue over a longer period; this is not confirmed by observations. The rate of growth was found to be of such a uniform character as to suggest that the curve of growth is fairly continuous rather than irregular. It seems to the author that the period of growth is not five years, a period given since Bierkander, but probably requires four years from the egg to pupation. The early (small) stage larvae were never found feeding. It is stated that they

feed on organic matter in the soil. The medium and large stages were almost invariably observed feeding (with the exception mentioned above in the case of the larger stage), and it seems that they feed continuously, save in the months of December to February inclusive. This observation was obtained from regular examination of potatoes placed in the breeding pots. Feeding is usually active by March and seems to decrease at the end of November. It is very difficult to starve these wireworms. Of 10 large wireworms placed in sifted soil without food November 3rd, 1914, nine were present January 22nd, 1915, and seven on March 8th, 1915. The three missing ones had probably been eaten by the others, as traces of the skins were found. These seven larvae had thus existed in soil free from any vegetable matter visible to the naked eye for 125 days; of the seven larvae only one remained on May 4th, 1915. In a duplicate experiment under similar conditions, 10 larvae lived from November 3rd, 1914, to February 11th, 1915, i.e. 100 days. The larvae will tolerate a large amount of moisture, and will exist for at least a week in water-logged soil. On the other hand, wireworms kept in soil not supplied with water and allowed to dry up will die after a couple of days. It is hard to explain their independence of vegetable growth (such as pasture grasses and various crops) for food, in any other way than by assuming that they feed on the organic matter in the soil. This would help to explain the usual occurrence of these larvae in the lighter soils plentifully supplied with organic matter. In several cases, small and medium sized larvae were found buried in the farmyard manure under growing potatoes. It could not be said definitely that the larvae were feeding, but the condition of the mouth parts seemed to indicate that they were.

The usual depth at which these wireworms are found in the soil varies from one inch in pasture, to eight or nine inches in potato fields. In winter they bury themselves much deeper, and were found as much as two feet deep in the large drainpipes previously described.

The larvae bury themselves in soil surprisingly quickly, though their rate of progress on the surface is comparatively slow.

Eighty-three larvae had buried themselves at least two inches deep in soil, of about the same consistency of that in a potato field, in less than twenty minutes. On being exposed to light, they immediately travel in the opposite direction to the source of the light. They travel slowly, but fairly easily on an unpolished surface, and often progress carrying the abdomen bent in a slight curve to one side. This is one of the difficulties that hinder accurate longitudinal measurement.

4. Description of the Larva.

The full-grown larvae of *A. obscurus* measure about 19 mm. × 2 mm. (Plate XVI, figs. 1 and 2). They are semi-cylindrical and their colour varies from pale yellowish white, to yellow brown. The young stages are of a paler colour, which darkens as the larvae become older. The freshly-moulted larvae are easily distinguishable by their pale yellow white colour. The junctions of the body segments are of a slightly darker shade than the rest of the body, and are in addition marked with faint longitudinal striae (Plate XVII, fig. 6). The spiracles are placed on darker chitinised areas. The muscular impressions of the ninth abdominal segment are strongly chitinised dorsally and ventrally, and slightly so laterally. The legs are of a darker brown colour.

The head is approximately as broad as long, with the anterior margin of a dark brown, rounded at the angles. On each side of the head, just behind the antennae, but slightly more towards the median line, is a slightly raised, irregularly rounded, pigmented eyespot. Immediately behind each eyespot is a long slender seta. The antennæ are short (Plate XVII, fig. 10), each consisting of three segments. The first segment is stout, brownish round the base, with a clear area round the summit. The second is dark brown, longer in proportion to its breadth, narrower than the preceding segment, and somewhat enlarged on its outer and upper margin where it bears two stiff upright hairs. The third segment is dark brown, about one half the dimensions of the second segment, and bears two terminal lobes which are slightly lighter in colour than the second segment. The lobes of the third segment, when seen from above, consist of a long narrow lobe (almost as long as the second segment) flattened at the apex, and a smaller lobe placed ventrally to the larger lobe and nearly half as long as, and a little thicker than, the latter. The third segment bears, in addition, two setae. The head region bears two pairs of setae on the lateral margin, placed near the anterior and posterior edges. No attempt is made to discuss the homologies of the head region.

The mandibles (Plate XVII, fig. 7) are of a dark brown colour, irregularly flattened dorso-ventrally, and sickle shaped. The apex is somewhat blunt, and just below this is a blunt tooth on the inner margin. This blunt tooth is common to the genus *Agriotes* according to Schiodte (16). A second tooth that is more pointed than the former, projects nearly half way down. Just below, is a well-marked process (*la*) projecting inwards which is the "lacinia mobilis." Mangan (11) in describing the

mandibles of *Periplaneta australasiae*, quotes Hansen (*Ann. Mag. Nat. Hist.*, 6, vol. XII, 1893), as recording the "lacinia mobilis" as occurring in certain Coleoptera. The mandible articulates with the head, by means of the condyle (*co*) and the ginglymus (*gi*).

The first maxillae (Plate XVII, fig. 8) are strongly chitinised around their outer margin, and are of a uniformly brown colour. They possess a distinct cardo (*ca*) into which the chitinised margin continues, swelling into a small dark area. There is no definite distinction between the stipes (*st*) and the terminal lobe of the maxilla. The stipes bears, on its outer margin, two long thick setae and three shorter ones, all close together, and placed rather more than half way up. The maxillary palp (*mp*) consists of four joints, of which the first is broad in comparison with its length, the second nearly as broad but longer, the third narrower and shorter; all three segments are brown, darker at the edges, with clear areas at their summits. Of the three segments, each bears setae; the first, two irregularly placed; the second, two at the summit and two on the outer margin; the third, two on the summit. The fourth segment is short, rounded terminally, and of a light brown colour. A galea (*ga*) and lacinia (*la*) are present in each first maxilla. The galea is short, and composed of two joints; both being about as long as the first two segments of the maxillary palp. The terminal segment bears four short, slightly-twisted setae. The lacinia is not plainly differentiated from the galea, and bears a large number of closely-packed, slender, waving setae.

The labium (Plate XVII, fig. 9) consists of the basal mentum, which is brown, and slightly chitinised at the lateral margins. It bears, at the margin on each side towards the base, a long curved seta, and another on each side towards the apex of the labium.

The mentum articulates with the palpiger, which bears a two-jointed palp on each side. The palpiger is shield-shape, and of a dark brown colour, darker at the sides, and lighter in the median area, and with a clear space across the anterior margin. It bears two long setae placed anteriorly. The first joint of the labial palp swells out towards its extremity, and bears a whorl of small setae, commencing about half way up from the inside, and progressing outwards and upwards. The second joint of the labial palp is rounded, and appears to possess a flattened, pale area at the tip.

Between the two labial palpi is the ligula (*li*) which bears a short seta on each side. The further setae, much smaller than the previous two, are placed at the base of the ligula.

On the underside of the head, adjacent to the first maxillae, is a dark, strongly-chitinised ridge, diverging anteriorly and converging posteriorly. A dark line runs parallel, close to the preceding ridge.

The prothorax has a curved furrow running down each side, and is nearly equal in length to the meso- and meta-thorax taken together. The hairs on the body of the larva are arranged in a definite manner, which seems to be fairly constant. The first eight abdominal sterna each possess three pairs of hairs, arranged symmetrically along the margins of these sterna. On examining the larva in its lateral aspect, it is seen that the first eight abdominal terga, considered up to their mid-dorsal line, possess two lateral pairs of hairs; of which the posterior pair is placed near the margin, a little to the left of the plane of the anterior pairs, which are placed nearer to their margin than the former. Placed mid-laterally, and just over each spiracle (of the first eight abdominal segments), is a pair of short hairs about half the length of the other hairs. In a line with the spiracle and in the same plane as the two posterior hairs, is a single hair of similar length to the paired ones. The ninth abdominal segment bears, on the base of the pseudopodium (or anal papilla), two pairs of short hairs. There are nine pairs of spiracles, the first being thoracic (Plate XVI, fig. 2, *st*), and placed ventrally. The remaining eight pairs are laterally placed on the first eight abdominal segments anteriorly in each segment. The ninth abdominal segment affords a means of distinguishing the genus *Agriotes*; Perris (14) describes the typical anal segment of the genus as follows: "Dernier segment assez-longuement demi-ellipsoidal, terminé par une pointe, ayant de chaque côté, près de la base, une cavité de l'apparence d'un grand stigmate."

This applies truly to the larva of *Agriotes obscurus*, but requires to be added to. The anal segment is terminated by a slight constriction, which swells out into a blunt point, outlined in black (Plate XVII, figs. 5 and 6). The cavity, that Perris describes as being like a large stigma, is not a respiratory opening, but is possibly a muscle attachment[1]. The walls of the cavity are black, and thickened; only slightly at the sides, but strongly dorsally and ventrally. In shape it is oblong, with the anterior and posterior walls rounded. It is placed very high up, and near the base of the segment. The stigmata are oblong, with their dorsal and ventral walls closely apposed, and strongly chitinised; their median line points forward and upwards, save in the case of the meso-thoracic stigmata, where the median line points forward and

[1] Henriksen (8) also speaks of this structure as an "eye-shaped muscular impression."

outward. The ninth abdominal segment is equal to the length of the seventh and eighth segments combined, and possesses a ventral papilla or pseudopodium. This, according to Sharp (17), "represents a body segment and is generally described as being the protruded termination of the alimentary canal." The pseudopodium is round, sloping gradually to meet the eighth segment, and with a sudden slope posteriorly to meet the remainder of its segment. The apex is white and flattened and bears a few short hairs. The pseudopodium is of some assistance to the larva in walking. The three pairs of legs are provided with spines and are dark brown, short and terminating in a curved claw. The legs do not require any special description; the right leg of the third pair is figured in Plate XVII, fig. 11.

5. Description of the Pupa.

Before proceeding to describe the pupa, some details will be given with regard to pupation, etc. The first pupa was found on Aug. 14, 1915, pupation had therefore occurred within seven days, as the last previous examination had taken place on Aug. 7, 1915. At the previous examination it was observed that several larvae were very sluggish, and subsequent events showed that they were preparing for pupation. The first adult *Agriotes obscurus* was observed in a freshly emerged condition on Aug. 30, 1915. This would place the pupal period at from two to three weeks. Observations on other pupae show that this period is correct. Pupae were found up to the end of September, and all the adults had emerged by the 9th of October. Pupation takes place in an earthen cell at a depth in the soil varying from three to twelve inches. The pupal cell is oval and allows ample space for pupation. The average dimensions of three cells was 5 by 14 by 7 or 8 mm. The photograph (Fig. 1) shows a section through a typical pupal cell, with the pupa lying in a characteristic position.

The internal walls of the pupal cell are smooth, and appear to have been slightly cemented together with some glutinous secretion. The cell will stand air-drying without crumbling away, to a greater extent than an artificial cell made of similar soil but not cemented. The method by which the data concerning the pupal cell were obtained is as follows:—the plant pot, taken into the laboratory and the zinc cover removed, was filled level to the top with soil of a known depth, the soil being slightly compressed to produce cohesion. A piece of stout cardboard was placed firmly over the top and the pot then inverted so

as to stand upside down on the cardboard. The cardboard was gently removed, and the soil in the pot loosened by pressing slightly through the drainage aperture. The pot was then lifted up with a twisting movement; a cast of the interior of the pot was left. This cast could easily be examined with regard to the depth of any specimens in the pot, as the depth of soil added just previous to examination was known. With this method, pupal cells in perfect condition were obtained without trouble. The pupa (Plate XVI, figs. 3 and 4)

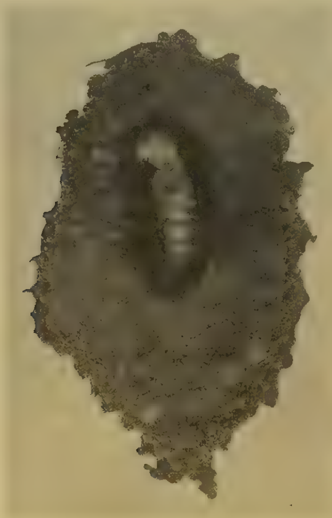

Fig. 1. Pupa of *Agriotes obscurus* in earthen cell. ×2. (Phot. J. T. W.)

measures up to 13 mm. long by 3 mm. broad, gradually tapering in the abdomen to a breadth of 1 mm. The general colour is white, speckled on the apices of the abdominal segments with small patches of rusty brown colour. The thorax is nearly as broad as long, and swollen. Two spines project from the anterior angle of the thorax, which is provided at the posterior margin, on each side of the middle line, with a small protuberance. The long, spatulate, posterior corners are each provided with a curved thorn-like structure, of a brown colour and pointing outwards. The third to the sixth abdominal segments are

slightly tooth-shaped at their lateral posterior margins. There are nine abdominal segments, of which the ninth is provided with two spines. Each spine possesses a small spur at the base. According to Curtis (6), these spines are movable. The ninth abdominal segment is provided ventrally with a flap, broad at the base, suddenly narrowing half way down the segment, and continuing in a smaller piece with parallel sides, which terminates in two sharp points. Beling (2) describes the pupa of *Agriotes obscurus* in a similar manner to the above, and adds that the segments of the antennae are as long as broad, that the wing cases reach to the middle of the fourth abdominal segment, and that the third pair of legs reach to the middle of the fifth abdominal segment. In the pupa described above, the third pair of legs only just reach on the fifth abdominal segment, and do not stretch half way.

6. Comparison with Larvae of closely allied Species.

The evidence showing that the common wireworms taken by the author in Cheshire and other districts are larvae of *A. obscurus* is as follows:—

All the larvae were examined under a binocular microscope, and were apparently all similar. At this time I was inclined to believe that the common wireworm represented the larval stage of *Agriotes lineatus*. A comparison of my material with Schiodte's figure of the anal segments of *lineatus* (16) did not solve the problem. The larvae undoubtedly belong to the genus *Agriotes*, and agree in general with the figure of *lineatus* given by Schiodte, but differ as to certain details.

Seven larvae at last pupated and the six adults that emerged were *Agriotes obscurus*. This seems to be corroborated by the interesting observations of Newstead (previously mentioned), where the area covered was mainly Cheshire, and part of Lancashire, and the records of adult Elaters devoured by birds give *A. obscurus* far more commonly than *A. lineatus*. As the occurrence of wireworms is admittedly universal, at any rate in this country, and as their attacks are not epidemic, these facts seem to point to *A. obscurus* as being the parent beetle of the larva in question. The identification of the larva provides the next problem.

The author has been unable to procure an undoubted specimen of the larva of *A. lineatus* for comparison, so reference was made to the literature. It was found that there is a scarcity of good accounts of the larvae belonging to the genus *Agriotes*. Some of the older accounts were

incomplete or even wrong, as Perris (14) quotes Bouché (*Naturg.* p. 186), as describing a larva of an *Athous* or *Corymbites* as *A. lineatus*. The best accounts of Elaterid larvae were found in Perris (14), Schiodte (16), and Beling (2), from which sources the comparisons are procured. The distinction between the genus *Agriotes* and that of *Elater* seems fairly clear. The possession of a blunt tooth, on the internal margin of the mandible (Plate XVII, fig. 7), indicates the genus *Agriotes* according to Schiodte, while Beling gives the presence of the chitinous cavity on the base of the ninth abdominal segment as indicating the genus. Beling describes *A. lineatus* and *A. obscurus*, as follows:

(1) Larve, sehr fein und seicht punktirt, fast glatt, blass braunlich gelb. In erde, vorzugsweise auf Aeckern....*Agriotes lineatus*.

(2) Larve unregelmassig seicht gerunzelt, starker und dichter punktirt, auch etwas dunkler als die vorhergehende gefärbt, mit derselben angleichenorten lebend....*Agriotes obscurus*.

Beling further says that the larva of *Agriotes ustulatus* Schall. is similar, but is smaller. He adds that the larva of *A. obscurus* seems more strongly and thickly hairy (punktirt) and wrinkled than *lineatus*; but characteristic differences remain to be found. His description of the *lineatus* larva would apply equally well to that of *A. obscurus*.

The position of the muscle attachments on the anal segment seemed to hold out a hope of identification, as the one illustrated by Schiodte differed in position from those observed by the author. This is unfortunately of little account, as the method of preserving the larva affects the position. If the larva is simply killed by being immersed in alcohol, it usually contracts, and the wrinkling of the skin and the position of the muscle attachment, shown in Schiodte, are obtained. Boiling the larva, and then passing it through different strengths of alcohol, preserve it naturally. Neither the muscle attachment placed so closely to the base, nor the wrinkled skin as figured by Schiodte, is ever seen in living or properly preserved specimens. There is a difference, however, between the pseudopodia. Schiodte figures the pseudopodium proper (as distinct from its base), as exhibiting three divisions, the third probably pertaining to the non-chitinous and fleshy tip. In the larvae examined by the author, there were never more than two (including the fleshy tip). As there is no reason to suppose Schiodte's figure is incorrect, this difference of the pseudopodium must be taken as specific. The mandible figured above (Plate XVII, fig. 7) differs from that of Schiodte.

A number of mandibles (both left and right) were examined and

were found to agree substantially with the figure given. Schiodte, however, figures the blunt tooth near the apex of the mandible as very slight, while the author's figure shows it to be more pronounced.

Furthermore, the position of the spiracle and hairs on the eighth abdominal segment, as represented by Schiodte, does not agree with the condition observed by me in the larvae of *A. obscurus*. These differences are constant in the specimens examined during this work[1]. The only other description to which I have had access is that of the larva of *Agriotes pallidulus* Ill. by Schiodte. The dimensions of this larva, 9 mm. long by 1 mm. thick, and its intense yellow colour, together with its anal segment, which is rounder than those of *A. obscurus* or *lineatus*, prevent confusion with either the larvae of *A. lineatus* or *A. obscurus*.

It would seem, that if the above differences between the larvae of *A. lineatus* and *A. obscurus* do not apply generally, then one is possibly a variety of the other, as it was formerly believed (Curtis, *loc. cit.*, p. 159).

7. Natural Enemies.

It would be natural to assume that the wireworms, on account of their underground existence, are fairly immune from enemies that prey upon them. In spite of this, they are subject to the attack of a number of persistent foes; particularly various species of wild birds. Wild birds are accountable for the destruction of vast numbers of insect larvae. This is especially so during the nesting season, as "practically all birds except doves and pigeons feed their young on an animal diet, whatever may be the character of the food of the adult" (9). Again, as Collinge remarks—"It should be remembered that the nestling season is also that when the destruction of injurious insects is most needed—i.e., at the period of greatest agricultural activity and before the parasitic insects can be depended upon to reduce the pests" (4 and 5). At present, definite information as to the food of nestling birds is somewhat scanty; but according to Collinge, the

[1] Owing to Mr Ford's departure to Rome he was unable to consult Henriksen's paper (8) before he left England. The Danish entomologist mentions that the larva of *A. lineatus* is "brownish yellow, faintly rugose and punctulate, mostly rugose." With regard to that of *A. obscurus* he adds "dark brownish yellow. On each side, between tergum and sternum a pale longitudinal stripe. Punctulate and with few rugae." I may add that the longitudinal stripe is not evident in any of Mr Ford's specimens. Henriksen does not appear to have reared any examples of *A. obscurus* from the larva to the adult. A. D. Imms.

Starling, Thrush, and Blackbird seem to feed Elaterid larvae to their nestlings as usual constituents of their diet. To quote one example—that of the Starling (Collinge, *loc. cit.*)—the food taken to one nest throughout an averaged period of half a day, required 146 visits and included 13 wireworms (species not stated): this was in the middle of May. It is probable that a large number of Elaterid larvae (as well as other pests) are thus disposed of during the nestling season. With regard to the food of adult birds more information is available, and it is possible to give a rough list of birds that are of importance as destroyers of Elaterid larvae, for "the frequent occurrence of these insects, and especially of the parent beetle, is very marked and goes a long way to prove that they form part of the regular food supply of various species of birds" (13).

The most useful in this respect are Plovers (or Lapwings), Gulls, Rooks, Jackdaws and Starlings, all devouring both adult and larval stages of Elateridae. In districts in proximity to the sea, Gulls are very beneficial—they are usually present where land is being ploughed; and they follow the plough, in numbers, searching for insects that are turned up. In one instance (Newstead, *loc. cit.*), forty-five *Agriotes* larvae were found in the crop of a Black-headed Gull (*Larus ridibundus*). Over the country generally, the Plover (or Lapwing) is one of the most beneficial birds to the farmer—in spring, its habitation is usually ploughed land. It undoubtedly destroys many injurious insects.

The Rook is not quite so wholly depraved as it is thought; for though it certainly takes a fair amount of seed corn, yet wireworms form about 9 per cent. of its food (Leigh (10)), and the young are fed on Elaterid larvae (Newstead, *loc. cit.*). Jackdaws and Starlings, both as nestlings and adults, feed on both larvae and imagines of Elateridae.

Curtis (6) states that the Sparrow preys on wireworms, but, up to the present, there is apparently no evidence available that might mitigate the uselessness of this ever-present pest. The author has little definite information regarding the particular proportion of the food of birds formed by the larva of *Agriotes obscurus*.

Wireworms appear to form a fairly constant constituent of the food of the common Mole (*Talpa europaea*), in fact insect larvae and worms form the greater portion of its diet. In an investigation on the food of moles (19) it was found that out of 100 moles, 41 of the stomachs contained wireworms. Though this investigation only extended over a short period (from Dec. 5, 1913, to Feb. 5, 1914), it yet serves to indicate

the value of moles from the economic point of view. Digestion in the stomach of the mole is apparently very rapid, thus rendering identification of the stomach contents difficult. It is, at any rate, certain that the animal is a very voracious feeder as Scheffer(15) found that *Scalops aquaticus*, a mole distributed over the eastern region of the United States, will consume more than its own weight of suitable food in a day. In the stomachs of nine moles which I examined it was found that three stomachs contained remains of Elaterid larvae (the remainder of the food was mainly various insect larvae, earthworms and slugs). It is interesting to note that in the districts where these nine moles were trapped, one farmer would not allow moles to be trapped, save when they were becoming too plentiful; on all the surrounding farms the moles were trapped regularly. The district has been examined for wireworms, and it was found that on the farm where the moles were protected, wireworms were very scarce, and then could only be found in small numbers in a very old pasture. On the surrounding farms, where moles were trapped, wireworms were taken in numbers. The animal is often a nuisance in grassland and cornfields, but it should be remembered that the mole is burrowing after food, and not for amusement. Again, as its food consists principally of insect larvae, and, as Adams(1) remarks, the mole does not hibernate, but continues active all the year round, it can well be seen how useful the animal is. These facts together with its voracity, and its need for meals at frequent intervals (Adams, *loc. cit.*), show the utility of the animal. It certainly destroys large numbers of wireworms, and should therefore be merely prevented from becoming too abundant. According to Graf(7), an important enemy of adult Elaterids is to be found in the Carabidae, species of *Calosoma* being particularly important in the control of *Limonius californicus* (sugar beet wireworm). Curtis (*loc. cit.*) mentions that the Carabs *Pterostichus madidus* and *Nebria brevicollis* are enemies of the wireworm. While collecting between 1914 and 1915, Carabid larvae and small adults (*Nebria*, sp.) were commonly found in potato drills, where wireworms were attacking the tubers. The adult Carabs most commonly found were *Pterostichus madidus* and *Nebria brevicollis*. It was found, as the result of carrying away two wireworms and a *Nebria brevicollis* in a tin box containing earth, that the Carab attacked the wireworms. On examination, after the day's work, the *Nebria* was alive, and the two wireworms dead and mutilated. Under natural conditions, no Carabs were seen attacking wireworms, though injured larvae were seen.

Wireworms with their tough highly chitinised skins, and underground habits, are almost free from internal parasites. Curtis (6), however, says that wireworms have a Hymenopterous parasite and quotes Bierkander as having found them. Forbes (*18th Rept. State Ent.* p. 47, 1891—2) records a parasitic fly as having been reared from a wireworm. Out of over 300 larvae collected, not one attacked by an internal parasite has been seen. Graf found the same result with 10,000 larvae of *Limonius californicus*. In this connection, I am informed by Mr Fryer that he has not infrequently found wireworms parasitised by what is probably a Proctotripid, at all events a Hymenopteron, but has never succeeded in breeding it out. No bacterial or fungoid disease was seen in any of the larvae in the field, or in the laboratory. In this connection, Graf reports as having occasionally observed fungoid attack in the field, but says that one fungus and two bacterial diseases were common under laboratory conditions. He also says that one bacterial disease was very fatal to the young wireworms, while the older stages seemed to be immune.

According to Leaflet number 10 of the Board of Agriculture (1916), wireworms are parasitised by a species of the fungus *Isaria*.

The pupae are difficult to rear; any disturbance or any slight variation in humidity or temperature, was sufficient to cause them to perish, and from the same causes some pupae passed into the perfect state but failed to mature. No disease of any kind was noticed. Graf notes that a few pupae of *Limonius californicus* were killed by a fungus.

Graf records a fungoid disease as having caused the death of adult Elaters under natural conditions.

Wireworms seem to be little affected by physical agencies under experimental conditions. They were found to be little affected by excess of moisture, but they could not resist dryness and were killed by frost. Under natural conditions it is probable that these factors are of no importance, as the larvae can always burrow down deeper in the soil, out of the way of frost, or in the search of greater humidity.

8. SUMMARY OF GENERAL CONCLUSIONS.

1. This paper contains an account of the larval and pupal stages of the Elater, *Agriotes obscurus*, whose larvae, together with certain other related larvae, are known as "wireworms."

2. The common "wireworm" in Cheshire, North Staffordshire, and South Lancashire, is the larva of *Agriotes obscurus*.

3. The life of the larva is probably four, rather than five years.

4. The larva of *Agriotes obscurus* can be distinguished from that of *A. lineatus* by means of the development of the blunt tooth, just below the apex of the mandibles (more prominent in *A. obscurus*); by the orderly arrangement of the body hairs, and the more anterior position of the stigmata; and by the pseudopodium or anal papilla on the ninth abdominal segment of *A. lineatus* having three apical divisions, while that of *A. obscurus* only has two.

5. Birds, particularly the Plover (or Lapwing), are of great value in checking the larvae of *Agriotes obscurus* (as well as other related species). The Plover, which is wholly beneficial, should be stringently protected. The common Mole (*Talpa europaea*) is also of great value in checking the pests, and should not be wantonly destroyed, unless increasing in too large numbers. The amount of damage caused by a mole is probably very small in comparison with the amount of good it does.

6. The larva pupates in an earthen cell in the ground, down to one foot deep; the pupal period is about three weeks; the imago remains resting motionless in the pupal cell for roughly two months, after which it comes to the surface, and hibernates under stones, clods, etc., until the next season.

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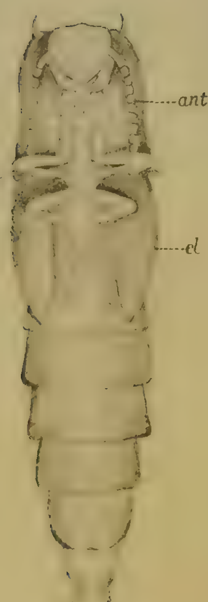

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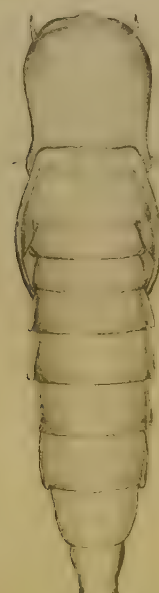

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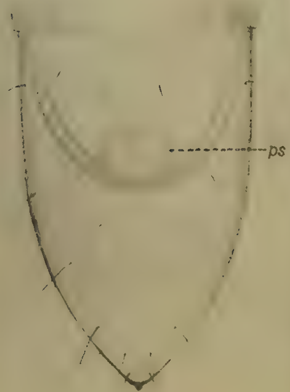

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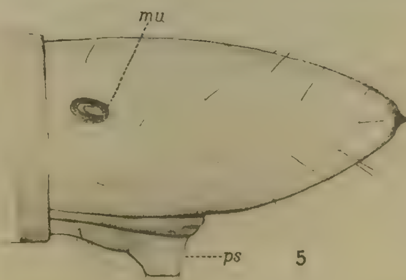

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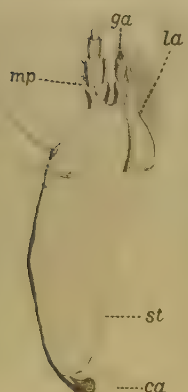

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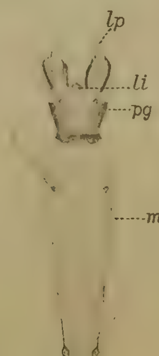

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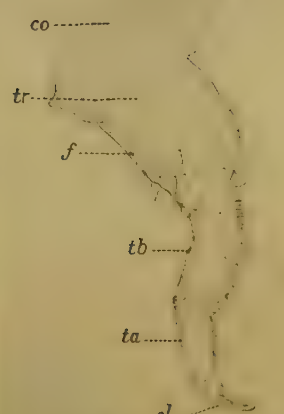

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DESCRIPTION OF PLATES.

PLATE XVI

FIGURE

1. Fully grown larva of *Agriotes obscurus*, lateral aspect, drawn from an extended spirit specimen. *ant.* antenna; *mu.* muscle attachment; *st.* spiracle; *ps.* pseudopodium. × 8.
2. Mature larva, as above, ventral aspect. *ps.* pseudopodium; *st.* first spiracle. × 8.
3. Pupa of *Agriotes obscurus*, ventral aspect. *ant.* antenna (left); *le.* elytron (left). × 8.
4. Pupa of same, dorsal aspect. × 8.

PLATE XVII

5. Lateral aspect of the ninth abdominal segment of the larva of *Agriotes obscurus*. *ps.* pseudopodium; *mu.* muscle attachment. × 24.
6. Ventral aspect of the ninth abdominal segment of the same. *ps.* pseudopodium. × 24.
7. Right mandible of the larva viewed from above. *gi.* ginglymus; *co.* condyle; *la.* lacinia mobilis. × 50.
8. Right first maxilla of the same viewed from below. *ca.* cardo; *st.* stipes; *mp.* maxillary palp; *la.* lacinia; *ga.* galea. × 50.
9. Labium of same, viewed from below. *m.* mentum; *pg.* palpiger; *lp.* labial palp; *li.* ligula. × 50.
10. Right antenna, seen laterally. *dl.* dorsal lobe; *vl.* ventral lobe. × 100.
11. Right leg of third pair of the larva. *co.* coxa; *tc.* trochanter; *f.* femur; *tb.* tibia; *ta.* tarsus; *cl.* claw. × 49.

ON THE BIOLOGY AND ECONOMIC SIGNIFICANCE OF *TIPULA PALUDOSA*.

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Part II. Hatching, Growth and Habits of the Larva[1].

(With Plates XVIII—XX and 3 Text-figures.)

The most common species of Crane-fly larva occurring in grass and corn land in the north-east of Scotland is *Tipula paludosa*. *Tipula oleracea* occurs also, but is much less frequently met with. Along with these, there has also been found in comparatively small numbers in fields the larval stage of *Pachyrhina histrio*, but this species appears to occur more frequently in garden ground. The following Tipulidae in addition have been found in the winged stage in the district surrounding Aberdeen:

Tipula varipennis, common and generally distributed.
T. gigantea, in small numbers.
T. lutescens, in small numbers.
Pedicia rivosa, L. widely distributed in the northern area, but not common.

The Egg.

Hatching of the flies goes on during the months of June, July, August and September, and as already recorded (Part I) the first mating and oviposition may take place within a very short period. In captivity, hatching mating and oviposition have all occurred within a few hours. A newly hatched female contains considerably over 400 shelled ova. In two such taken at random the actual numbers were found to be 448 and 490. A third female captured out of doors *in coitu* contained 255 black shelled ova together with a quite small number—about 12—of pale coloured immature shelled examples. The form of

[1] The work recorded in this series of papers has been carried out with the aid of Grants from the Board of Agriculture for Scotland.

this female when found indicated that oviposition had previously taken place. A female *Pachyrhina histrio* taken in the open contained 259 black shelled ova.

The egg measures 1·1 mm. × ·4 mm.; it is black in colour, with a dark purplish metallic lustre. As development proceeds this lustre diminishes, and finally before hatching the shell is of a dull black colour. The covering of the egg is a strong tough membrane, which is completely formed around the egg before copulation takes place. I have been unable to detect a micropyle, but this may be present. It is possible that the membrane before coming in contact with the air is permeable to the spermatozoon. The somewhat remote possibility of parthenogenesis taking place with the first brood of eggs has not been overlooked, and females have been kept apart from males from the period of their hatching until death, but oviposition never took place under these conditions.

The Early Larva.

The emergence of the larvae takes place in about 14 days after the eggs have been laid. They are then of a pale reddish sandy colour, and about 2·7 mm. in length, Plate XIX (*b*). When fully extended, thirteen body segments can be made out. On each of these from the third to the twelfth there is a small tuft of laterally placed, moderately strong bristles. The thirteenth segment which bears the spiracles and terminal papillae, has a pair of tufts of relatively stronger and longer curved bristles, borne alongside the large lateral conical para-anal papillae. These tufts constitute the most striking difference between the early larva and the later form.

Through the skin the two longitudinal tracheal trunks are visible, and also the alimentary canal with its four anteriorly and one posteriorly placed diverticula. The masticatory apparatus is well developed, both mandibles and first maxillae being strongly toothed.

In a short time the segmental bristles tend to become very short or worn away, and so also do the anal tufts. Traces of the lateral bristles persist even in the fully formed "grub," but the anal tufts disappear completely. These changes appear to come about by attrition. In about 12 to 13 days from the time of hatching the larvae are 4—5 mm. in length when fully extended, and already resemble the older and more familiar "leather jacket." By about three weeks they have attained a size of 6 mm. They feed from the first day onward.

The fully grown Larva: External features.

Owing to the difficulty of keeping alive recently emerged larvae which were prevented from burrowing into the soil, it has not been possible so far to follow the changes in external form effected at the various moults. The larva when fully grown attains at full extension a length of about 40 mm. It is now of a brownish-grey earthy colour interspersed with irregularly placed blackish dots. Frequently the longitudinal tracheal trunks, two in number, may be seen through the skin. The shape is cylindrical, slightly narrowed anteriorly, and expanded posteriorly into a peristigmatic papillae-bearing area. The skin, which is generally tense in healthy larvae, exhibits the following characteristics:—along each side there is a moderately wide strip which on the animal contracting folds outward, forming a pair of blunt keel-like longitudinal ridges. Besides numerous transverse wrinklings, there are slight but definite transverse furrows marking off distinct segments. Eleven of these can generally be counted. Each segment bears on its ventral surface four very minute bristles, two lateral and two near the middle line slightly in front of the lateral pair. On the first four segments behind the head dorsally, there is a row of bristles, and a pair of dorso-lateral bristles on succeeding segments. The head bears a pair of short jointed antennae; there is a very strong chitinous and highly complex mouth armature which includes massive toothed mandibles with palps, a pair of serrated first maxillae, notched united second maxillae and elaborately folded and bristled labrum (Plate XIX (*a*)). The whole set of structures is based upon a large strong bivalved chitinous support which surrounds the gullet.

The anus, which is sub-terminal, is surrounded by large fleshy lobes and a pair of large retractile laterally placed conical papillae. Beling regards these papillae as characteristic for this species.

On the somewhat truncated terminal region there is a pair of large brown coloured circular stigmata, each with a lighter glistening dull golden marginal ring. This stigmatic area is expanded on its border into six conical papillae, of definite form and arrangement. There is a ventral pair whose tips are black with a clear central area. This pair appears to have a sensory function, and may be seen at times in the living animal apposed to the stigmata above. Below each of these ventral papillae there is a pair of small pigmented spots which are sometimes united to form a short streak on each side. The remaining

four papillae project dorsally in two pairs. These bear on their exposed surface numerous fine hairs which follow the boundary of an elongated slightly raised conical area; the outer pair is tipped in black. Fig. 1.

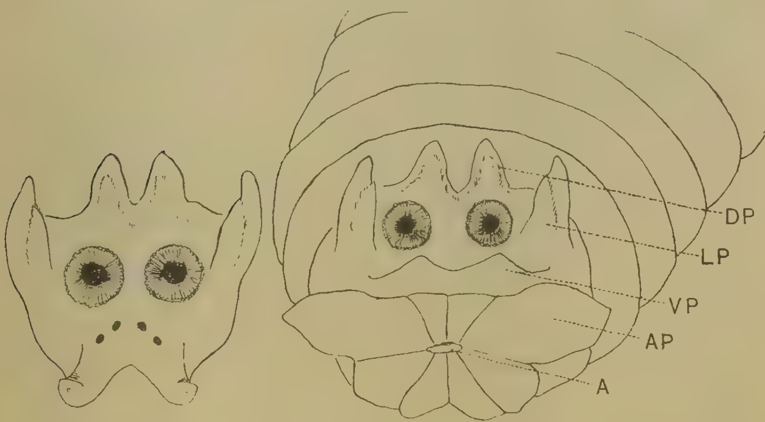

Fig. 1. Stigmatic area in *Tipula paludosa*, (*a*) showing papillae with hairs, stigmata, and pigment spots; (*b*) showing anus and fleshy lobes, together with para-anal papillæ.

DP. Dorsal papillæ; LP. Lateral papillæ;
VP. Ventral papillae; AP. Para-anal papillæ, outside stigmatic area; A. Anus.

Duration of Larval Period.

In the N.E. of Scotland the adult flies may be seen frequenting cultivated land from early in June to the beginning of October. It appears to be the accepted opinion in England (Theobald, *Agricultural Zoology*, 1913, p. 228) that there are two generations of these flies, *T. paludosa*, and *T. oleracea*, in the course of the year. Our observations have shown that in this area, probably owing to the higher latitude and more rigorous climate, there is only one.

The following observations made upon a small collection of flies reared from eggs which hatched in September, 1913, are typical of the results obtained in rearing during several years. The parent flies had hatched out within small laboratory cages or had been caught upon the college farm and placed in these. The cages had wooden roof and

base, wire gauze sides and glass front. They contained a bed of turf two to three inches deep. Mating and oviposition readily took place, and on the turf being broken up later a considerable number of recently hatched larvae was found. A later search in the month of October, however, showed the mortality to have been considerable. On 1st November, the turf was again broken up and the surviving grubs collected and measured. The lengths were taken by allowing the larvae to crawl upon a sheet of paper and pricking this at the moment of their maximum extension. About one half the number of larvae was found to be under 16 mm. in length, and the remainder from 30 to 35 mm. It is remarkable that the larvae may attain to nearly their maximum length quite early, but it must be noted that they are relatively slender at this period. Subsequent growth takes place in the direction of thickness. By the end of the month the disparity in size was not so great. They all conformed to Beling's description of *Tipula paludosa* larva. In November clover was sown in the cages; this for a time afforded opportunities for feeding but was allowed to die out during winter. These larvae lived throughout the greater part of the following summer in soil containing decaying vegetable matter only, and duly pupated and emerged as adults in July and the early days of August.

As a control upon the above, portions of second year's grass upon the farm were dug up in October, the turf was disintegrated, shaken up in sieves, and the soil searched. Larvae were found and measured in a similar manner, and the commonest size at this date was found to range from 20 to 25 mm. These also were identified as *T. paludosa*.

Larvae collected out of doors during November and the first half of December showed a maximum size of 20 mm. These were relatively slender at this degree of extension, and contracted when handled to much smaller bulk than the laboratory reared specimens.

The larvae collected out of doors on 1st November, and which were separately caged, but kept under similar conditions, when fully extended measured 36 mm. in December, but in this state were distinctly more slender in the body. Examination of the contents of the alimentary canal showed that they had fed upon the decaying rootlets in the soil. As the season progressed it was found that there was eventually no significant difference in size between those reared indoors and those living a natural life outside. The records of soil temperature taken on the farm showed that there had been little frost during this experiment. The winter was a mild one, and the facts suggest that some feeding at least had taken place amongst the larvae out of doors. In a subsequent

season caged larvae kept short of food were found in February to measure from 20 to 30 mm. Generally, there has been found out of doors great variation in the size of larvae at the end of winter in the same district and even upon the same field.

Tipula paludosa has been kept under direct observation throughout its whole life cycle, and owing to variations in the length of the larval stage pupation and consequently hatching of adults is spread over a considerable period, viz., in this district, June to September. (Rarely, I have found adults in the cages in May.) Under experimental conditions of limited food supplies larvae have been kept alive and been continuously under observation for fifteen months. The minimum duration of the larval period has been found to be about nine months—September to June. Before all the larvae of a season have pupated the next season's larvae may have appeared, so that there may be larvae present in the soil all the year round. There is a possibility that this fact may have led to the view that there are two generations of flies in the year. I have had under observation in breeding cages in the month of July, larvae, pupae, and adults of one generation, together with developing eggs and emerged larvae of the next generation—all alive at the same time; and in the variable climate of the region under observation such occurrences are not improbable in the field.

Bionomics of the Larva.

The newly emerged larvae are very susceptible to drought, and when kept in dry soil were found to die off quickly. Strong sunlight, even when the soil is moist, was also fatal in a short time. Artificially reared larvae require to be kept moist and sheltered from direct sunlight, otherwise the mortality in the early days is very great. Larvae which are hatched from eggs which have been placed upon the surface of the ground immediately burrow into the soil, avoiding the light. A large proportion of larvae reared from eggs in 1913 died in the course of the first eight weeks, especially towards the end of this period, notwithstanding all attempts to reproduce natural conditions, and only a comparatively limited number of flies have been reared from many thousands of eggs laid in the laboratory cages.

In view of the fact that very large numbers of eggs are laid, and of the probability that the adults only rarely approximate to these numbers, there must be a considerable mortality in the course of the life-history, due, of course, to various factors. Our experience suggests that the first

few weeks of life constitute a period in which the insect is particularly susceptible to the prevailing physical conditions. While difficulty was experienced in rearing large numbers of larvae from the egg to the adult stage, no such difficulty was met with in rearing flies under the same laboratory conditions from larvae collected in the field in late winter. It may be suggested for example that wet weather in the end of summer, and early autumn months, will favour the survival—apart from natural enemies—of greater numbers of larvae, and that conversely, prolonged drought will tend to kill off numbers of those hatched about this time. In this connection it may be worth while to quote the opinion held by some farmers in this area that a wet summer and autumn foreshadows a plentiful supply of crane-fly in the following year.

The Larva on Farm Lands.

Published references to the activities of the larva as far as I have been able to trace them deal exclusively with instances of serious or even excessive damage effected by these insects upon grass or corn crops. But in the course of the present investigation it has become clear that Tipula larvae are very commonly present upon farm lands, sometimes in considerable numbers, without their presence becoming apparent. Cases of excessive damage have also been experienced, but the following instance may be taken as an average experience under the conditions named. It is quoted in full because it illustrates a number of features related to the larval habits.

These observations were made in the spring and summer of 1913, upon the College farm at Craibstone. Search was made for the presence of Tipula larvae in the end of March and beginning of April. The weather was cold at the time, and the searches were not very fruitful. The Woodlands field (Fig. 3) which was in grass at this time was selected for enquiry, samples being dug up at a number of places, and the turf thoroughly examined, but no Tipula larvae were obtained. Grey slugs were particularly plentiful. This was on 3rd April. On the 19th, ploughing was in progress and the plough was followed, samples of the furrow were taken, disintegrated, and searched, but no larvae were found in this way. Further search by two observers resulted in four larvae being found. These were found under stones at the surface, on the part not touched by the plough. After the field had been sown and rolled it was again examined on the 29th, and larvae were now found to be very numerous under the turf clods upon the surface. In

the interval between the times of examination there had been a good deal of rain.

On the 3rd of May the field was visited at 6 a.m. The two previous days had been dry and sunny, but in the end of April there had been much wet. The morning was fine, and at 6 a.m. the sun's rays had reached the western end of the field only. The eastern end was still in the shadow of the trees.

A search was commenced at the eastern end where there was some frost in the ground. In about 45 minutes 94 larvae had been collected. In the southern hollow where the sun had now reached, 42 were obtained in about 10 minutes; on the crest of the field at the west end (in sun) 75 were found in 20 minutes, and on a low part (N.W. corner), in the sun, 15 in 15 minutes. Two collectors were at work. In all in about $1\frac{1}{2}$ hours 226 larvae were obtained. In some cases from six to a dozen were found in a single piece of turf. The smallest number appeared to be present in the shade at the highest level of the field (E. end). The larvae were found mostly under the turf clods, and largely in the "mids"; sometimes they were lying on the soil below, and sometimes embedded in the turf with heads well buried amongst the roots. They were not seen distributed generally amongst the sown grain.

The field was again examined on the 10th of May. There had been continuous and heavy rain for several days previously, and the ground was very wet. The oats had "brairded" early in the week, but the wet weather had rendered rolling impossible. At the time of search rain was not falling, but there was a mist close down to the ground. Larvae were frequently found beneath the loose turfs upon the surface, and they were also to be seen crawling freely on the ground. Some trouble was taken to find them in the act of attacking the young crop, but with no success. The ground in a number of places was scraped with a toothed digger and the plants turned over. A few larvae were found in the ground in this situation, i.e., free from turf clods and amongst the soil in which the oats were growing, but none was seen actually attacking any part of the crop. The oats were scarcely an inch above ground. In many places no corn could be seen and here the ground was turned with the digger. A few larvae were found in this way, but it could not be said that they were more numerous than in other situations. It was found that mostly the seed in these places lay deeper and was growing all right.

During the following week the weather improved and there had been several warm and dry days. On the 17th the field was again

examined. It had been rolled during the previous day. The crop looked well, and there were no indications of Tipula attacks. Larvae were again found in the most usual situation, viz., below the turf clods; 40 were collected in a few minutes. Search amongst the growing oat plants resulted in only one larva being found; none was seen upon the surface, and none detected attacking the crop.

On the 21st another examination was made. The weather in the interval had been showery, but not very cold. The day was warm and there was some wind. The field was carefully searched, particularly for traces of larvae moving freely in the soil or actually attacking the crop. They were found in the usual places below or burrowing into loose "foggage" upon the surface. In a few cases they were found below flat stones at the surface. Only a very few were obtained by searching the open soil around the oat plants. In this search the soil was turned over with a digger and the oat plants uprooted. Sometimes the ground was scraped and stirred. The examination ought to have discovered larvae if they were present in the soil in proximity to the roots of the oat crop, and it is concluded they were absent in this situation at the time of search, viz., between 10 a.m. and 12 noon. The weather at the time was warm and showery, and larvae could always be found amongst the decaying turf. The crop at this date, notwithstanding the undoubted presence of Tipula larvae in large numbers, showed no bad effects. Up to the beginning of July, when the last search for larvae was made upon this field, there was no apparent effect of the presence of Tipula upon the crop. On this occasion the search was effected by cutting the crop over certain areas, and sifting the soil by spade and sieve. Larvae were obtained, but no pupae were seen.

It should be mentioned that in view of the presence of Tipula in appreciable numbers and the possibility of an attack ensuing, a plot experiment was early arranged upon the field with the object of testing the effect of rolling and of some common manurial substances. The experiment, though negative in its results as far as its original purpose is concerned, is given here because it confirms the conclusion that Tipula was not visibly damaging the crop. The field, which is surrounded by trees, was rich in humic matter. Below is given a diagram of its situation, and of the experimental plots together with the report of Mr W. Findlay, N.D.A., Superintendent of Field Experiments.

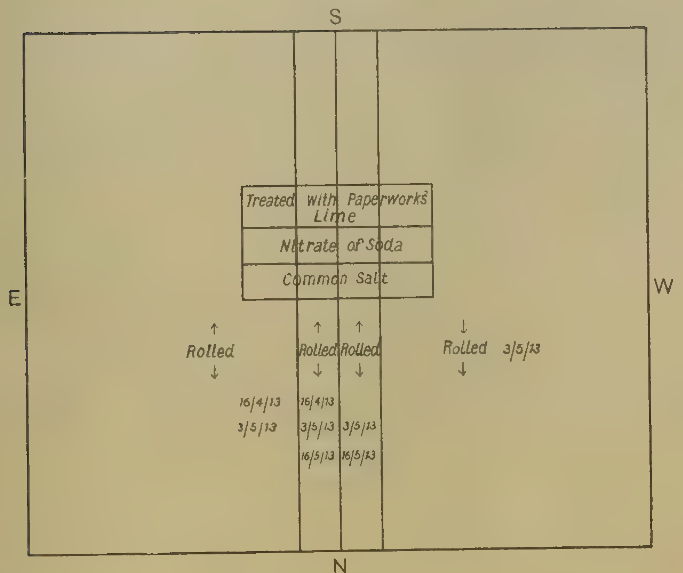

Fig. 2. Diagram of Experimental Area of Field.

Report. The Woodlands field at Craibstone was ploughed out of Lea during the second week of April, 1913, and immediately sown with Sandy oats at the rate of six bushels per acre. A heavy rainfall prevented the whole of the field being rolled at that time, and an interval of about two and a half weeks elapsed before the soil was dry enough to finish the operation. One strip the whole length of the field was rolled three times, and other two strips were rolled twice.

On the 21st May three plots were laid off and treated as follows:

1. 4 cwts. Paperworks' Lime per acre.
2. 1½ „ Nitrate of Soda per acre.
3. 2 „ Common Salt per acre.

The accompanying plan will show the scheme of the different treatments and cultivations.

At no time was there any difference either in the thickness or strength of the crop between the parts rolled at different times.

The plot to which Nitrate of Soda was applied showed an increased crop of about 20 per cent., but those to which Lime and Salt were applied could not be distinguished from the rest of the field.

WM. M. FINDLAY.

The experience here recorded has been general for a series of years; large numbers of Tipula larvae have been regularly obtained from lea fields upon farms on Deeside and elsewhere in the neighbourhood, which during the periods tested had no crop losses due to their attacks. In a good many such cases the numbers obtained from single fields were considerable. A case where serious damage was effected is given upon page 132.

Fig. 3. Plan of Craibstone Home Farm.
Experimental Field, No. 5.

Summarising the outstanding features of this record we note: (1) An apparent scarcity of larvae in spring before ploughing took place. The failure to find Tipula was not as subsequent finds proved due to their smallness of size, and it does not seem likely that they were deeply situated in the soil at this time. They have rarely been found below six or eight inches under the surface. Tests made with deep cages in winter (Feb. and March) yielded only insignificant numbers (eight per cent.) below six inches from the surface.

(2) When known to be present in the field they could not be found in the act of attacking the crop. The suggestion that they feed at night is plausible, but observations on larvae in cages have shown that they feed readily at all times. The fact that there was an abundance of humic matter in the soil is probably not without significance in this connection (see pp. 127—8).

(3) The occurrence of most of the larvae at the surface beneath loosened decaying turf. Their presence here in the spring is general; they occur both loosely below, and also very frequently deeply embedded in the turf. The disturbance of the soil in ploughing and harrowing is probably the cause of their gathering in these situations, and their presence is probably primarily due to the need for shelter and moisture.

(4) The absence of harmful effect upon the crop notwithstanding their presence in considerable numbers throughout the spring and summer. This feature is considered in connection with the further data given below.

Further consideration of feeding habits.

With a view to rendering clearer the feeding habits of the larvae and to throw light upon the circumstances under which they attack growing crops numerous experiments were made of which the following are illustrations.

I. A small lot of larvae reared from eggs laid in September was kept in ordinary field soil covered with loose turf in a small laboratory cage. No crop was sown in the soil, but it was watered from time to time. They wintered under these circumstances and continued throughout the following summer. The larvae pupated in July and the last of them emerged as flies on the 4th August and mated on the same day. The life cycle in this case occupied about eleven months.

II. A collection of larvae was kept out of doors in small boxes containing ordinary garden soil without growing vegetation during the months of May, June, and July. They survived this treatment, but were undersized. Some managed to pupate, but others died in the larval stage. One imago was observed to fail in the act of emerging from the pupal case. The larvae of this group did not on the whole do so well as those of lot I, and the mortality towards the end of the experiment was high. Dissections showed the presence of vegetable fibres in the intestine, and a considerable amount of gritty material. It may be mentioned that this latter is normally present in the intestine.

III. A collection of larvae was kept in small cages with no growing plants, and a limited amount of decaying vegetable matter amongst the soil. These conditions were maintained during the months of May and June of the present year. At the end of June all were alive and healthy looking, and some were well grown. The cages were set in a large field rearing box containing washed sea sand, amongst

which the metal cages containing the larvae were sunk. A considerable number of larvae wandered from the cages and were subsequently recovered amongst the sand, alive and to all appearances quite healthy. Adult flies were later seen emerging from the cages in the laboratory to which they were removed during July. The larvae of this and Experiment II were collected in the fields and had wintered out of doors.

The foregoing, together with other experiments and observations of a like nature have shown clearly that the Tipula larva may subsist in the soil and complete its development independent of the presence or absence of a growing crop upon the ground. The results here obtained have led to the institution of further and more exact experiments dealing with the nutrition of soil larvae including Tipula. These are at present in progress.

IV. A number of larvae caged in the autumn of 1915 were kept in soil without growing vegetation except for a short period when a small quantity of corn sown in the cage germinated. In February they were found to have reached a fair size. Several killed and examined on the 19th were found to contain soil particles and fragments of vegetable fibre. At this date they were found mostly in compact earthen cells formed against the sides at the bottom of the cage. This habit has been frequently observed in winter and suggests a quiescent period under the adverse conditions of cold, confinement and restricted food supply.

In order to determine more clearly the circumstances inducing destructive attacks upon crops the following type of experiment was resorted to:

Groups of larvae were put up in large cylindrical glass cages of about 10 inches diameter, in prepared soil, in which the visible amount of vegetable matter was very slight. This soil was, further, mixed with well washed sea sand. Around the cylinders between the glass and the soil, oat seed was introduced. The cages were kept at room temperature and were examined daily. After the corn had germinated, the larvae were kept under close and continuous observation for prolonged periods at a time. The larvae appeared sluggish, and not much movement was seen in the day time, although their burrows soon became very numerous between the soil and the glass (Plate XX). They could be seen lying in these, and after the corn had germinated, or even before this, they could be seen attacking it, gnawing at the

husk, radicle and plumule. They were also seen eating the blades which had come above the ground. The glass cylinders had removable ends of perforated zinc of fine mesh. A few larvae passed through the perforations at the bottom although these were small Within the cage they tunnelled freely to a depth of six or seven inches. After a week, when all the corn appeared to have germinated, and both radicle and plumule were of some length, the cylinders were removed and the state of the seedlings ascertained. These were separated out carefully and placed in water. The soil was removed as far as possible by gentle washing, and each seedling examined in turn. Care had been taken that no other creatures were present in the soil capable of damaging the oats. There were usually about 25 larvae present. A typical result is given:

34 seedlings apparently sound.
11 had radicle or rootlets more or less eaten away.
39 had plumule cut through or bitten into. This includes a number attacked in the blade, above ground.
8 seeds were attacked. In some cases this had been effected before germination, in others, afterwards. In some of these the food store was completely eaten out, in others only partially so.
92

Equal to 63 per cent. of the plants damaged.

The factors contributing to such a result appear to be:

(1) The absence of decaying or other vegetable matter, with consequent restriction to the crop for food supplies.

(2) The large proportion of larvae to the cubic content of soil—two dozen to about $1\frac{1}{2}$ cubic feet of soil.

(3) The confinement of the larvae to a limited amount of space; it was not possible for them to leave the cages.

A further factor to be noted is the favourable conditions of warmth and suitable moisture, inducing rapid germination. This would be of some importance in the field in favour of the crop "growing away" in a proportion of cases before the damage done became irremediable.

In other experiments of this nature with germinating oats, clover, or timothy grass, the numbers of larvae used were very much greater, with correspondingly serious damage. It was found subsequently in examples of very severe attacks upon crops in the field, that the numbers used in these experiments were in excess, and that serious loss may ensue where the numbers, in average samples of an affected area, are about 10 to 15 per square foot of surface (see p. 133).

Migrations.

The use of large numbers of larvae upon experimental plots or open shallow cages brought to light the fact that scarcity of food in spring due to over stocking results in the larvae migrating. The first proof of this was obtained from a lot of 120 larvae contained in a small box in soil in which clover had germinated. As the food supply became exhausted the larvae left the box in large numbers and were found crawling about the laboratory. Two other instances of this occurred in connection with field plot experiments at Craibstone. In these experiments, small plots of $2\frac{1}{2}$ feet by 6 feet were walled off with wooden boards sunk six inches in the ground and standing about three inches above the surface of the soil. It had been established in experiments in a previous winter that these larvae even in very cold weather rarely went below this depth of six or seven inches, and it is not considered likely that they burrowed beneath the limiting boards of the plots. The plots were stocked with large numbers of larvae; in one instance three adjacent contained 900 larvae each and in other three plots, there were 700 each. When it was discovered that the crops in these had failed, at the end of June the soil in each was sifted for larvae. The hatching season had just commenced, but only a very few flies had been seen; of the 2100 larvae in the second group of three plots only 295 were recovered, together with a small number of pupae and two or three empty pupal skins.

Another plot was not disturbed. It had stood exposed in the same way as its neighbour, for some weeks, but later a large field cage was fitted upon it. Here there had been placed originally 2700 larvae, and now to these were added the 295 recovered from the other plot. In due course flies hatched out and appeared in the cage, but the numbers up to the end came very far short of those of the grubs introduced. The soil was searched and no trace in the form of empty pupal cases or dead larvae was found.

In a duplicate experiment on Holm Farm, in Lewis, carried out at the same time, designed to test the relative resisting or recovering power of selected oat varieties, it is probable that a similar migration took place. This experiment consisted of plots of Hamilton, Sandy and Potato oats, into each of which 900 larvae were placed. There was also a control plot of Hamilton oats, which received no larvae. The oats were sown on 18th May, and "brairded" on 3rd June,

The plots were under continuous observation till the end of June, during which time no difference was observed in the growth of the different varieties. At this date the plots were searched and only a few larvae could be found. The local observer states that he is confident birds did not interfere with the plots, and the only conclusion that can be drawn is that the larvae migrated from the plots before the crop had germinated. Had they remained and continued their development, pupae and empty pupal cases would have been found in the plots. As a matter of fact these were examined before adult flies had begun to appear. Had the larvae died within the plots it is certain that the remains of some at least would have been found, when the soil was passed through the sieve. But nothing of the kind was found.

The question remains—do they migrate in appreciable numbers normally, in search of food? On the fields under observation I have found them wandering upon the surface, but so far only occasionally. With regard to the plots, it is to be borne in mind that the larvae had been placed in excessive numbers on soil which did not contain any growing plants (except the ungerminated corn), while around there was abundant green vegetation. Their power to climb up even a few inches of a vertical board was not expected; that they did so was also evidenced by their being found in the control plots in which none had been placed.

A further interesting case, bearing on the point, is recorded in an editorial note in the *Scottish Naturalist*, 1915, p. 1.

The reference is brief and may be quoted in full:

"A few weeks ago it was reported to us that the inhabitants of a certain district in Perthshire had been seriously alarmed by an invasion —not of Germans—but of an immense number of small worm-like creatures which crawled over the road near the houses in such numbers as to make walking decidedly unpleasant. Examples of the creatures were brought to us, and were recognised as 'leather jackets'—otherwise the larvae of *Tipula oleracea*, the commonest species of crane fly."

As bearing further on this subject, the Editorial note proceeds to quote from an article by P. Desol, in *Compt. Rend. Soc. Biol.*, Paris, LXXVII. No. 21, June, 1914, pp. 126—7.

The summary given is quoted from the October 1914 issue of the *Review of Applied Entomology* (Series A). As the conclusions here are at variance with my own on *T. paludosa* and with the occurrences just described they are reproduced. The observations were made in the meadows of Avesnois. "In the spring of 1911, circular patches of from 15

to 60 feet in diameter, or the entire areas of meadows, were to be seen covered with yellow and dead grass the roots of which were found to be full of the earth-coloured larvae of *Tipula oleracea*. Grasses and clovers are chiefly attacked while plants with hard or thick roots are not affected. Where the infested zone borders on a furrow the larvae fall into it during the night and being incapable of climbing out may be collected in large numbers. They are not migratory, so that healthy meadows may be found close to infected ones. Infection is due to chance circumstances bringing fertilised Tipulids to the ground." I am satisfied that the statement that the larvae are not migratory is erroneous and that the existence of healthy meadows near them may easily be otherwise explained.

The conclusion to the experiments just described—unsatisfactory as regards the object for which they were planned—was due to the excessive numbers of larvae used on the plots. The large numbers were decided on with a view to avoiding an indifferent result, and this was supported by field experience, where large numbers had been seen and which had not proved harmful. In its result, however, it is useful and the possibility of migrations from waysides, waste land, or grass fields to germinated corn crops cannot be neglected. The experience recorded on p. 122 may be recalled, in which a field examined in April, and found almost negative as regards the presence of Tipula larvae, was later found to have an abundant supply. This problem is under continued investigation.

Various field experiments and observations have been made with the view of testing the effect of manurial agents and various insecticides upon the larvae. One of these is quoted below, but in general the results obtained were not consistent, and in cases of some well-known insecticides were not corroborated by direct tests upon larvae in confinement. The experiments were useful mainly in bringing into prominence some factors which are contributing elements affecting the degree of attack upon the crop, and which are considered in the succeeding section.

Field Experiment: Holm, Stornoway. Oat Crop.

In the previous season the field was grazed by cattle right on to the end of November, and with sheep and cattle on to January. Ploughing began early in January, and was finished by the end of February.

Weather.

From the beginning of October to the middle of March it was exceptionally wet and boisterous. There was practically no frost and the temperature was normally mild. The Farm is worked on the five shift system; the present tenant came to the farm in 1910, and previous to this the farm had been much neglected, especially as regards drainage and weeds. Oats were sown in the last days of March. After sowing, the field was immediately harrowed—one single and two double. It was not rolled till 23rd May.

The oats "brairded" in about a fortnight from sowing time and looked strong and healthy for a week. About 20th April the crop began to look sickly in parts and by the end of another week the presence and activities of Tipula larvae became apparent. The weather for some weeks after sowing was warm and sunny.

The state of the crop was reported to the College authorities in May, and on 24th May arrangements were made to treat the field experimentally. At this time, it showed parts fair, parts very thin, and fairly large tracts were quite blank. All over the field leather jackets could be readily found, and on the bare parts they were present in very large numbers; scores could be found near the surface in a square foot of soil. A part of the field including the worst portions was plotted as under:

	Plot No. 5	Plot No. 4	Plot No. 3	Plot No. 2	Plot No. 1
Rolled twice					
Rolled once					
Unrolled	No manure	4 cwts. common salt	1 cwt. Nitrate of Soda	2 cwts. salt	1 cwt. Nitrate of Soda

The manures were applied on the 6th of June, and two-thirds of the total area rolled early the following morning. On the 14th June one half of that part was rolled a second time. The other third was not rolled.

On June 26th the larvae were still to be seen, and active. Ten trial counts were made on square foot samples taken at random. These were dug to a depth of six inches and the larvae counted. The following are the numbers found: 4, 5, 4, 4, 5, 4, 12, 14, 14, 13. No sample

tested was found blank. The last four lots were from Plot 5, unrolled part. Plots 1, 3, and 4, showed improvement on the crop at this date.

On July 14th the results showed as follows:

Plot 1. Originally the thinnest. Recovery good.
Plot 2. Originally 2nd thinnest. Now same as Plot 1.
Plot 3. Originally the best. Much ahead of all.
Plot 4. Originally only medium. Second best.
Plot 5. Originally 2nd best. Worst of all.

There was no very obvious difference between the once and twice rolled parts of the plots, but the unrolled parts were decidedly poorer. Larvae were still active in the field at this date.

On 15th September the field was visited and the crop as a whole found to be in a very good condition, it having tillered remarkably well. The variety of oat was Hamilton. Signs of the larvae having been present were scarcely apparent, any patching being extremely slight. The nitrate plots did extremely well, the salt ones also were very good. From the appearance of the crop in May, it would have been impossible to have foreseen such an excellent recovery. Local circumstances did not allow of the estimation of the yield of the separate plots by weight per plot.

Factors favouring the Larva.

It may be regarded as well established that Tipula larvae are generally distributed and ordinarily present in the soil, though not necessarily only upon farm lands. Even upon these they have been found to be present in appreciable numbers, when no recognisable loss in crops resulted. The question of importance is,—what are the circumstances determining a destructive attack upon a crop?

Naturally the first condition of importance is the presence of excessive numbers. The various factors contributing to the periodical appearance of large numbers of any species of insect have not hitherto been appreciated in advance, and indeed cannot be said to be well understood. In general with regard to Tipula they may be held to include:

(1) favourable weather conditions for the survival of the large numbers of young which are generally produced:

(2) unfavourable conditions for competitors:

(3) ready supply of food:

(4) absence or diminution of numbers of natural enemies.

As regards the influence of the weather in affecting numbers it has been already noted that the newly hatched larvae are very susceptible to drought and that the mortality in laboratory reared larvae which are not kept moist is high. There is a fairly general popular impression that severe winter with plenty of frost kills the larvae, but our experience so far does not bear this out. Larvae were left in the open exposed upon stones during a night of severe frost, and were found alive the following morning. The apparent beneficial result following frosts seems to be due to the improved tilth favouring a more rapid primary growth of the crop in spring. The environmental factors are the subject of continued investigation. Unfortunately, owing to the presence of the larvae on cultivated land, the food supply is adequate. Food is not ordinarily scarce, and as already indicated a high percentage of larvae kept in captivity complete their development upon ordinary turf in the soil.

The question of natural enemies is dealt with in a succeeding paper.

Tipula attacks on Oats.

Conditions unfavourable to the oat crop may render it susceptible, and Tipula, even when the numbers are not excessive, may work havoc. Apart from the question of manuring and general farm practice which are not considered here, the weather in spring has been found to be a significant factor. These observations have been carried on during a period of seven years; along with these the experience of over 130 farmers on a wide area has been collated and there is a universal testimony to the fact that a cold late spring in which the primary growth of the plant is delayed constitutes one of the most certain conditions for crop failure due to Tipula attack. It is during the early days of growth from the time of sowing until the adventitious root system is established that Tipula attack results in the destruction of the individual plant. After this period the plant may be regarded as out of danger; it may be weakened but probably it will not be killed outright by subsequent attack. Any cause therefore tending to extend the period of germination or immediately subsequent growth increases the liability of the crop to loss from Tipula attack. In this area the time between sowing and brairding for oats in an average season is 10 to 14 days. In a series of seven cases which came under observation in one season in which failure of parts of the oat crop, attributed to Tipula attack, took place, this

period ranged from 16 to 21 days. There was one case in which, owing to severe drought, it lasted six weeks. The actual proportions of crop failure were stated thus:

Period between sowing and germination	Proportion of crop lost
16 days	one-fourth of whole crop
6 weeks	one-third of whole crop
17 days	one-tenth of whole crop
3 weeks	loss incurred; proportion not estimated
16–18 days	almost one acre destroyed
3 weeks	one-third of crop
3 weeks	one-third of crop; it recovered later

On this account early sowing, especially in our north-eastern climate, is attended with a certain amount of risk. The same risk would apply to all late districts in seasons when fine weather early in spring tempts the farmer to sow early.

Tipula and Oat Varieties.

The question of the relative resisting or recovery power of different oat varieties in relation to Tipula attack has been investigated both in the field and in plot experiment. The experience of the farming community within the College area has been obtained and discussed, and the general view is given below. There exists some opinion that the larva exercises a selective capacity and prefers some varieties to others, as is evidenced by the following statements:

"They (i.e., the Tipula larvae) like Potato oats better than Sandy."
"Potato oats are more liable to attack than Red Oats or Sandwich."
"They are more fond of 'Potato' varieties."
"Waverley suffers from attack more than Potato or Sandy."
"'Banner' oats suffer more than Potato."

All the evidence, however, goes to show that this is not the correct interpretation. It is not the larvae which "prefer" particular varieties, but particular varieties which show better power of recovery from their attack. Some of the writers expressed their experiences more carefully:

"Of the 'Waverley' and 'Hamilton' oats which were sown side by side, the 'Waverley' had to be resown; 'Hamilton' oats were only slightly thinned."

"The 'Red Oats' resisted poorly, 'Providence' and 'Potato' did better."

Taken collectively the views of over a hundred farmers who have had experience of Tipula attack is contradictory with regard to the

PLATE XIX

(b)

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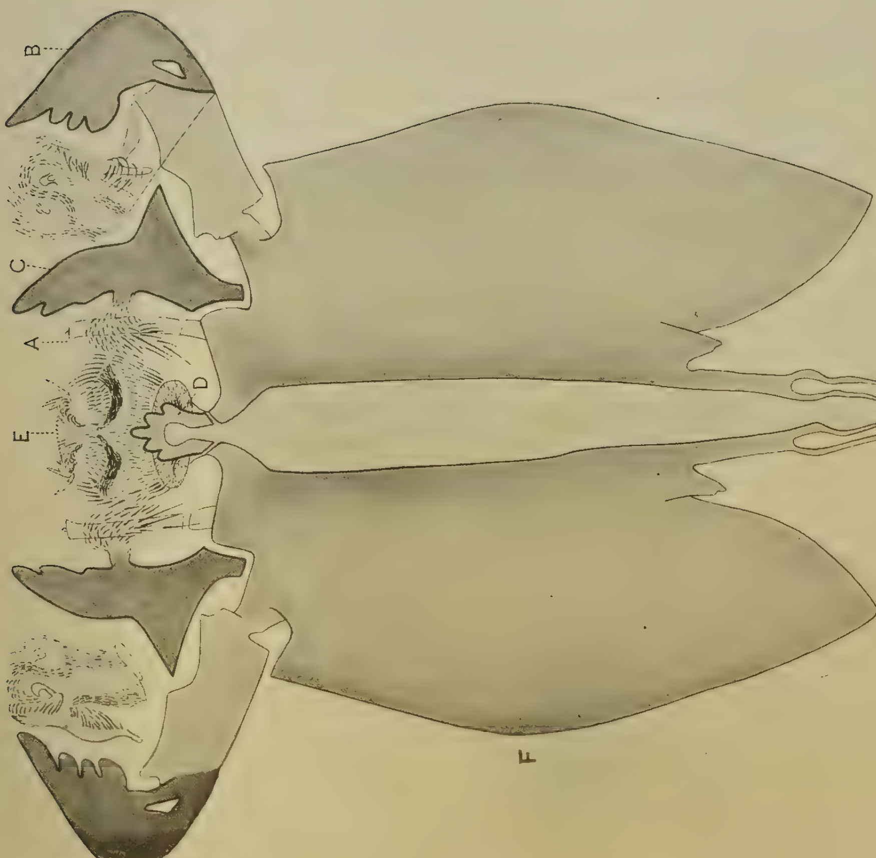
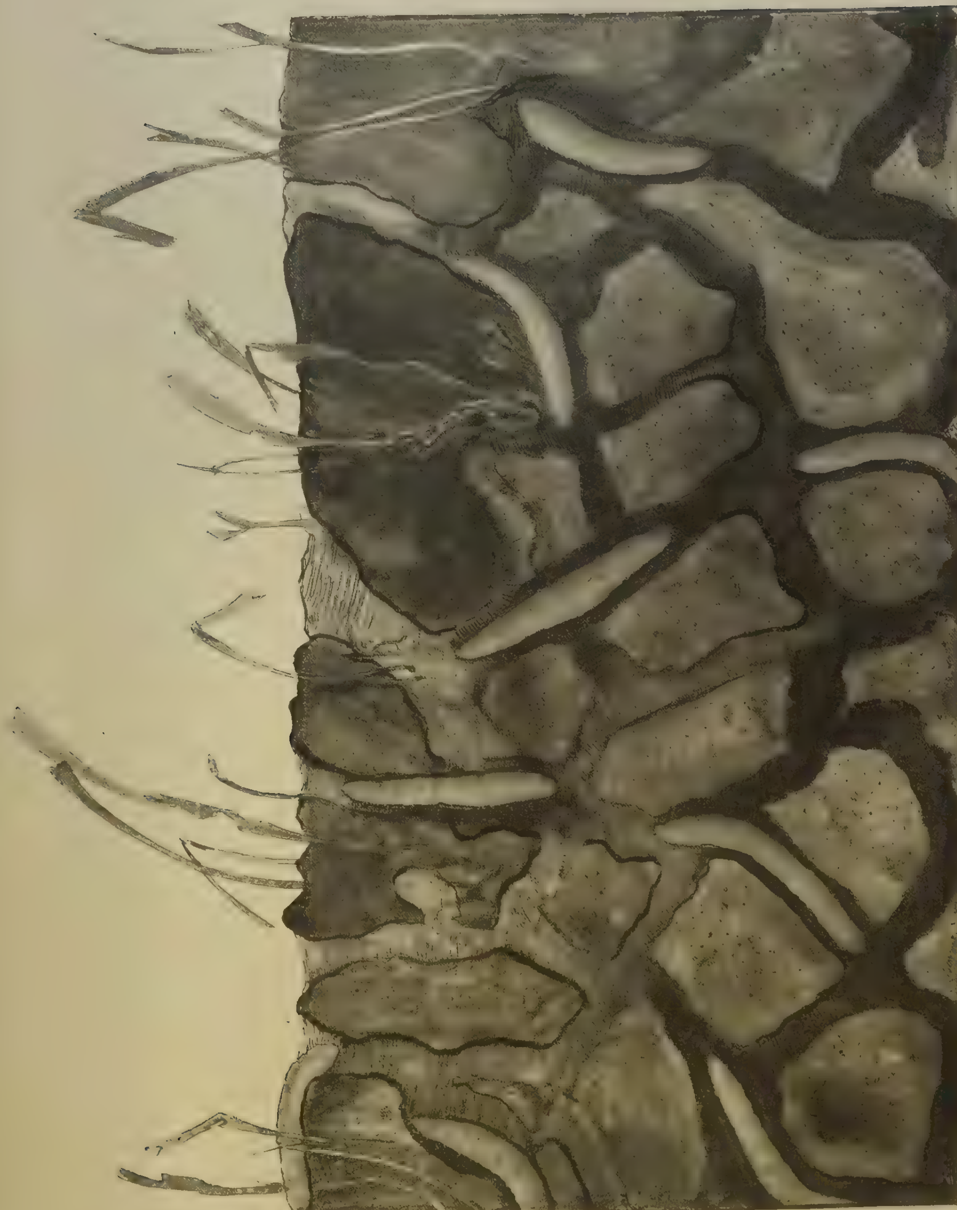


(a)

Del. E. Harvey



Del. B. Simpson

relative merits of particular varieties. This is likely to be the case so long as other factors such as quality and source of seed, type of soil, particular agricultural practice, manuring, and so on, are not considered. But there is general agreement that the newer varieties of oats have distinctly less power of recovery, because they do not tiller well. This is well borne out by wide experience.

Another practice which has been found of service in overcoming Tipula is to effect a "change of seed." Seed grown on the coast and sown in an inland locality, and seed grown in the Lothians and sown in Aberdeenshire have in both instances proved more resistant where larvae were at work, than native grown oats. Further, seed from an early district and sown in a late one, also from a light soil to a heavy one, have in both instances been proved satisfactory measures against losses from Tipula attack.

The subject of preventive agricultural practice and remedial measures in relation to Tipula are dealt with in a further paper.

REFERENCES TO PLATES.

Plate XVIII. *Tipula paludosa.* Larvae. 3 times natural size.

Plate XIX. *a.* Head armature of *Tipula paludosa.* (A) antenna; (B) mandibles; (C) first maxillae; (D) fused second maxillae; (E) labrum; (F) internal basal support of appendages.

b. Newly hatched larva of *Tipula paludosa.*

Plate XX. View of larvae in soil within glass rearing cage, showing their burrows and damaged oats, which were grown between the glass and the soil. Sketched from nature.

REFERENCE.

Beling, Th. Beitrag zur Naturgeschichte verschiedener Arten aus der Familie der Tipuliden. *Zool. Bot. Gesellsch. in Wien,* xxiii, Bd. 1873, pp. 575–592.

NOTE ON ATTACKS OF *PHYLLOTRETA VITTULA* ON SPRING CORN.

Phyllotreta vittula has recently been recorded by several continental writers as a serious pest of spring corn. It has also been recorded by Lind Rostrup and Ravn[1] as a serious pest of winter barley in Denmark. The following extract from Baranov's account of this pest in the Government of Moscow is taken from the *Review of Applied Entomology*, Series A, Vol. I, page 214. "This beetle becomes yearly more numerous and the damage done by it to the summer-sown crops is increasing. The author never found it or noticed any damage done by it on the winter-sown crops. It winters in the province of Moscow in the imago stage among the roots of summer stubble and of *Triticum repens*. It emerges from the earth with the return of warm weather (the middle of May in 1912) and immediately attacks the sprouting grain. It does the most injury at the time of pairing, and at the beginning of June its injurious activity decreases. It feeds on the epidermis and parenchyma of the leaves, forming channels in them parallel with the veins. If the number of channels is not great the leaves may recover. The author did not succeed in finding eggs of this insect. The harm done to the plants by the larvae was considerably greater than that done by the adults, and the author noticed that the former sometimes pass over to another plant. The plants attacked by the larvae generally die or are greatly retarded in their development. After the 15th June the insects decreased in numbers and by the beginning of July there remained only single individuals, but they reappeared on the 15th August in great numbers."

On April 27th, 1914, the writer received from Mr E. S. Beaven of Warminster, Wilts, specimens of seedling barley plants in which long strips were eaten between the veins of the leaves, very similar to the damage described above. With these plants were sent specimens of *Phyllotreta vittula* which were said to be present in large numbers and eating holes in the young barley plants soon after they come above the ground. The attack was not so severe as that described by Baranov.

[1] *Rev. App. Ent.*, Ser. A, Vol. III, p. 698.

This year a similar attack was noticed at the Rothamsted Experimental Station by Mr A. W. Rymer Roberts, who writes as follows: "On one of the fields at the Rothamsted Experimental Station I noticed during the course of last May considerable damage done by *Phyllotreta vittula* to young barley of three or four inches in height. The field had been ploughed out of ley the previous winter, and though not ordinarily one of the experimental fields, had this year crops of various kinds in strips. In addition to the barley, spring-sown oats on the next strip were also affected, though not so severely as the barley.

"The beetles usually seemed to attack the upper surface of the leaf first, eating through the parenchyma and leaving only the epidermis of the under surface. The holes so made, as you will see by the photograph I am sending, are in general oval, running between the ribs of the leaves, as Baranov describes; only in our case the pest was not bad enough to render the holes confluent, nor did I notice that plants were killed by it.

"The beetles disappeared about the end of the month, and I did not notice a second brood, though I may have overlooked them owing to pressure of other work.

"Though the damage done was not very material in permanent injury to the crop, in view of the seriousness of the attacks on spring-sown corn in Russia, it will be well to watch for any extension."

It will be noticed that the attack at Warminster was earlier than that at Rothamsted which occurred at about the same time as that of Baranov's. The attack in both cases occurred when the plants were very young, and therefore at a time when the plant is most likely to receive a severe check.

As this pest is so widely spread on the Continent, and has occurred at such widely separated places as Rothamsted and Warminster (where attacks would not easily be overlooked) it is possible that the damage done by this pest in other parts of England may have escaped notice.

F. R. PETHERBRIDGE.

SCHOOL OF AGRICULTURE, CAMBRIDGE
December 1916.